

I. THE MECHANISM OF TRANSLOCATION: METHODS OF STUDY WITH C¹⁴-LABELED 2,4-D¹

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INTRODUCTION

AN IDEAL HERBICIDE has long been regarded as one that would move from the foliage of weeds into the roots following application. Although in some situations it is preferable to treat weeds through the soil, such treatment involves possible reactions of the herbicide with the soil; even where such reactions do not occur, the dilution effects of the soil, which weighs over 3 million pounds per acre-foot, are an obvious disadvantage. Much time and effort have been put into the search for translocated herbicides, particularly for use against perennial weeds (Robbins, Crafts, and Raynor, 1942; Crafts and Harvey, 1949; Crafts, 1953a, 1953b).³

As discussed by Robbins, Crafts, and Raynor (1942), the acid-arsenical solution, sodium chlorate, and ammonium sulfamate, though translocated, left much to be desired. Introduction of 2,4-D changed the picture, for in this compound we have an organic chemical of extreme potency that moves readily through plants, apparently in association with food materials. And such are the selectivities of the cells and tissues of many plants that 2,4-D may be absorbed and moved through living cells of the foliage at concentrations that are lethal to roots. The treated foliage is not killed, at least not until enough of the toxicant has been moved to destroy whole root systems. In fact, use of excessive amounts of the chemical or inclusion of toxic additives in the spray solution may bring about such rapid killing of the foliage that movement into and injury of roots is lessened.

Not only is 2,4-D an excellent translocated herbicide; it is as well a useful translocation indicator for use in purely physiological studies. And 2,4-D carrying carbon 14 as a radioactive tracer is even more valuable. With the synthesis of 2,4-D carrying carbon 14, it seemed that the long search for an ideal translocation indicator was at an end, and that this complex function of plants would soon be completely elucidated. The following pages, however, will point out the great complexity of the problems involved and

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³ See "Literature Cited" for citations referred to in text by author and date.

some of the reasons why a complete answer is still lacking. It is hoped that they will, as well, point out substantial progress, and that they may serve as an inspiration to further research on the problem. While it should be obvious to any well-trained plant physiologist that the ideal chemical that will kill every weed to which it is applied may never be found, it is heartening that so much has been learned about the processes of absorption and translocation and the conditions that promote them. At least, as a result of the many studies being carried on, we are able to explain why many applications of translocated herbicides fail. And to a limited extent, by describing the ideal conditions for absorption and translocation, and by fitting formulations to the needs of these two essential functions, much improvement has been made in the practice, particularly so far as certain hard-to-kill species are concerned. Experiments described in this paper have contributed to some of this improvement.

Translocation of water, salts, and organic compounds in plants was one of the first functions to arouse the interest of plant physiologists. And although the mechanics of absorption and translocation of water and salts is fairly well understood, the movement of organic materials has been the subject of much controversy. Even at the present time, after the introduction and use of the hormone indicators and radioactive tracers, agreement has not been reached on the basic mechanisms involved in organic solute movement. The phloem is undoubtedly the principal tissue involved in rapid longitudinal transport, but whether movement of solutes and the solvent water together in a stream constitute the essential mechanism, or whether the solutes move independently of the water and of each other, is not agreed upon.

Final solution of this problem is important in two ways. Once the basic mechanism has been described, demonstrated, and agreed upon, effort now being expended in attempts to settle the controversy can be turned to studies on the nature of the translocation process in various plants, and the role of various factors in modifying it. Once the latter studies have been made, the results can be immediately applied in the practical use of translocated herbicides in the field.

As an example of the nature of this problem, if 2,4-D, after absorption, is moved with food materials in the so-called assimilate stream, then for effective killing of roots, application should be made after the spring flush of growth when replenishment of the root reserves has started but before meristematic activity in the roots has ceased, for, as van Overbeek (1947) has stressed, 2,4-D is most active against cells in the meristematic state. On the other hand, if 2,4-D translocation is independent of food movement and dependent only on 2,4-D gradient, then application would better be made during the spring flush of growth while meristematic activity throughout the plant is at a maximum.

Much of the evidence now at hand indicates that translocation of 2,4-D and similar compounds takes place via the phloem in the so-called assimilate stream. The present writer is in sympathy with this view, and many of the experiments reported in this paper were designed to clarify the mechanism and substantiate this theory. The forces responsible for the functioning of this mechanism have been described (Münch, 1930, 1943; Huber, Schmidt,

and Jahnelt, 1937; Huber and Rouschal, 1938; Rouschal, 1941; Crafts, 1951, 1953*a*, 1953*b*).

Briefly, the phloem is visualized as an extended and ramifying osmotic system in which exists, because of metabolic processes, a gradient, or gradients, of concentration of osmotically active substances (assimilates). Because the phloem system has a common source of water in the xylem, absorption of water in regions of high concentration where synthesis is taking place brings about increased hydrostatic pressure, whereas utilization of assimilates in growth, storage, and respiration results in lowered concentration and hence lowered hydrostatic pressure where these activities are going on. Because of the gradients of hydrostatic pressure so created, solution moves from regions of synthesis to regions of utilization through the sieve tubes of the phloem. And any solute present in this stream is carried with it. The accumulation of evidence that 2,4-D moves by such a mechanism in plants (Crafts, 1953*b*) substantiates this concept. Recent evidence that the protoplasts of the sieve tubes are plasmolyzable (Currier, Esau, and Cheadle, 1955) will have to be considered in any detailed visualization of such a mechanism.

METHODS

Many methods have been used in carrying on the studies. In the earlier tests, single-drop applications of 2,4-D solution were made to leaves of greenhouse-grown plants, and the expression of 2,4-D symptoms was used as evidence for the final distribution of the chemical. Later, the bean-bending test was used for studies on the role of pH, surfactants, and other factors in absorption and translocation of 2,4-D (Day, 1950, 1952).

With the provision of labeled 2,4-D, this material has been used as a tracer, and autographing has been the principal means of detection, though some counting has been done. Paper chromatography has also been used. Details of methods will be given with the description of experiments.

DETAILS AND RESULTS OF EXPERIMENTS*

Background Work

After comprehensive studies on translocation of 2,4-D in the bean plant were completed (Day, 1950, 1952), a study was undertaken of the distribution of absorbed 2,4-D in greenhouse-grown cotton plants of differing sizes and states of maturity (Clor, 1951). Treatment, in most cases, consisted of applying 0.01 ml of a solution of 2,4-D acid in 2 per cent ethyl alcohol to the center of a cotyledon or leaf. In general, the droplet was dry in 15 to 60 minutes.

From results obtained it seemed justifiable to assume that absorption of 2,4-D by different leaves was constant and that lack of appearance of symptoms on older plants was not caused by lack of absorption. When older plants were treated on different leaves, subsequent manipulation, such as pruning, which induced growth of axillary shoots, brought about expression of 2,4-D symptoms on these young growing shoots. As Gifford has since shown (1953), expression of 2,4-D symptoms results from changes in young leaves that

* The research reported in this paper represents the efforts of a number of students as well as of the writer. The credit due these workers is indicated as the results are reported.

are undergoing rapid differentiation at the time of treatment or immediately after. Leaves that are mature at the time of treatment are not affected, and after a certain period of response leaves subsequently produced may show diminishing effects. The duration and extent of 2,4-D injury to cotton depend primarily upon dosage, and low dosages may give only temporary response followed by complete recovery.

When these experiments were planned it was hoped that injury to roots from translocated 2,4-D would be measurable. It proved impossible to measure the immediate effects on growing roots; although large doses actually killed the roots, small amounts translocated from treated leaves caused no measurable injury. In two experiments dosages of 16 μg to cotyledons and first leaves produced swelling of the hypocotyl just above the root zone.

Briefly, Clor's studies showed that about 8 hours were required for absorption by cotyledons of cotton and translocation through the petiole to the stem; treatment of young plants (3 to 4 weeks old) resulted in movement both upward and downward from lower leaves; movement from upper leaves (third and fourth) occurred mostly in an upward direction; with older plants (6 weeks or more) movement from upper leaves was acropetal, and no movement toward the apex occurred from lower leaves; downward movement of 2,4-D from the upper leaves of old plants, as well as upward movement from lower leaves, took place when sinks for food utilization were created above or below the treated leaves, respectively.

The growth substance did not move downward when the stem was steaming below the treated leaf; it moved upward past a ring only when it was applied in high concentration. When the growth regulator was added to the nutrient solution it was readily absorbed by roots and carried to upper parts of the plant in the transpiration stream. Injury to the upper portions was proportional to the amount of 2,4-D added to the nutrient solution.

When cotyledons and first leaves of young plants were treated with 8 μg or more of 2,4-D, most of the substance moved down to the root and caused distinct swelling of the lower portion of the hypocotyl. When only the cotyledons of such plants were treated with 8 μg or more of 2,4-D, the substance moved to the root and leaked out to the nutrient solution where it was absorbed by the roots of untreated control plants growing in the same jar. The latter developed symptoms in their upper leaves about two weeks after the appearance of symptoms on the treated plants (fig. 1).

Clor's final conclusions from these experiments were that the movement of foliar-applied 2,4-D takes place primarily in the phloem and that the movement is dependent upon the movement of food materials in the plant and not upon the 2,4-D gradient. These conclusions are in agreement with Day (1950, 1952) and other workers in this field (Crafts, 1951).

Following Clor's work, a series of tests was conducted in the greenhouse,⁵ designed to clarify the relations of pH of the applied solution, formulation of the compound, and presence of surfactants and buffers to absorption and translocation of 2,4-D and similar compounds. Work with the dinitro herbicides had proved that acidification of the spray solution materially enhances the toxicity, and chemical reasoning indicated that association of the dinitro-substituted-phenol molecules increases their solubility in lipoids and hence their permeation of the cuticle (Crafts and Reiber, 1945). Similar reason-

⁵ Conducted by Emelio Levi.

ing applied to data on 2,4-D indicated that with this toxicant too, acidification of the salts or use of the parent acid should enhance the toxicity (Crafts, 1948).

To substantiate this reasoning, five experiments were run, using the bean-bending test, to find the effect of pH on absorption of 2,4-D and 2,4,5-T. All five tests and one subsequently performed^a were in agreement; between pH 10 and pH 2 there was a regular increase in the rate of absorption and the quantity absorbed. Of the six experiments, three used kidney bean plants, three used black-eyed peas. One involved 2,4,5-T, five were run with 2,4-D. Table 1 presents data from one of the tests. In this one, black-eyed peas

TABLE 1

ABSORPTION AND TRANSLOCATION OF 2,4-D AS INDICATED BY BENDING OF BLACK-EYED PEA PLANTS TREATED BY APPLICATION TO ONE UNIFOLIOLATE LEAF

pH of solution	Bending (in degrees) at end of:								
	1 hr.	2 hrs.	3 hrs.	4 hrs.	5 hrs.	6 hrs.	7 hrs.	8 hrs.	28 hrs.
0.5.....	0	0	1.0	3.0	1.5	2.5	7.0	10.0	11.0
1.0.....	0	0	2.0	2.5	1.5	1.5	4.5	5.5	7.0
2.0.....	0	0	5.5	21.5	65.5	100.5	102.5	94.0	91.5
3.0.....	0	0	4.5	27.0	58.0	88.5	94.0	87.5	92.5
4.0.....	0	0	0.0	15.0	54.5	94.5	96.0	81.0	84.0
5.0.....	0	0	0	11.0	46.0	87.5	100.0	88.0	86.0
6.0.....	0	0	1.5	12.0	44.5	90.5	100.0	91.0	79.5
7.0.....	0	0	0	1.0	9.0	32.0	70.5	56.5	82.5
8.0.....	0	0	0	1.0	1.0	10.0	24.5	72.5	76.5
9.0.....	0	0	0	0	1.5	10.5	39.0	70.0	91.5
10.0.....	0	0	0	0	1.0	12.5	40.0	68.0	86.5

(*Vigna sinensis*) were used; the dosage was 2.5 μ g of 2,4-D per treatment; the solution contained 0.1 per cent Trem 615 as a surfactant. Phosphoric acid and trisodium phosphate were used to buffer the solutions within the range covered by these compounds. Hydrochloric acid was used to obtain the pH values of 0.5 and 1.0.

As noted in the table, solutions of pH 0.5 and 1.0 were so acid that they produced rapid burning of the treated area. Evidently the cells were killed and translocation was inhibited. Though slight injury occurred at pH 2.0, it was not enough to inhibit absorption, and translocation was normal.

One notable exception to this pH relation occurred in an experiment where acetate, phosphate, and borate buffers were compared. Whereas a regular reduction in absorption occurred with phosphate-buffered solutions through the range 7 to 10, with borate buffer absorption was quite constant and about midway between that from phosphate-buffered solutions at pH 6 and pH 7. Evidently borate ion in some way enhances the uptake of 2,4-D through the cuticle. Figure 2 shows the relationship of these two absorption rates.

It has been recognized for years that inclusion of a surfactant in a 2,4-D solution increases its effectiveness. To confirm this view and to find the relation of pH to this enhanced uptake, 2,4-D acid and the sodium salt of 2,4-D were compared with and without surfactant. In this case Trem 615

^a Conducted by Charles McCarthy.

was used at 0.1 per cent. Figure 3 presents the results of this test. There seems to be no question as to the increase in 2,4-D uptake when a surfactant is used, and apparently the effect is present with the sodium salt (pH 8.0 at the concentration used) as well as with the acid (pH 3.3 at the concentration used).

Our next experiment tested the relative effectiveness of several concentrations of two surfactant formulations, Trem 615, a nonionic polyhydric alcohol with ester linkage, and Multifilm L, a mixed formulation containing a hydrocarbon fraction and free and combined fatty acids. Figure 4 shows that between the concentrations of 0.05 and 0.8 per cent there was little difference in effects. In all subsequent tests a concentration of 0.1 per cent has been used for the surfactant content of 2,4-D test solutions.

Five screening tests on commercial surfactants were run in 1950 and 1951, four by Levi and one by McCarthy. Out of some 17 materials, Nonic 218 brought about the most rapid absorption of 2,4-D acid in two trials; it equaled an experimental material in one trial; and it was inferior to several materials in two trials. Since a nonionic surfactant is desirable to use in experiments with 2,4-D acid, because it has no effect on the pH of the solution, Nonic 218 has been selected for use in subsequent studies. Other nonionic surfactants with similar properties would probably serve as well. From much testing of surfactants it seems clear that they vary widely in chemical properties (Snell, 1949), in wetting ability (Currier, 1954), and in toxicity to plant tissues. And they are extremely variable in their effects on 2,4-D absorption—so much so that it is impossible to rate any number of them in the order of their effectiveness because successive experiments give differing values. Nonic 218 was used in many of the tests to be described because it has a definite composition (not blended), it is one of the most effective tried, it is low in toxicity, and it is nonionic and relatively non-reactive.

The 2,4-D molecule can be combined into a great number of compounds having widely differing properties. It has already been shown that the acid is more effectively absorbed than the sodium salt. According to chemical reasoning (Crafts, 1948), the alkyl esters should also be readily absorbed, but their partition from the cuticle and other lipoid fractions of the leaf is questionable. A great number of alkylamine and alkanolamine salts have been used. Again, the balance of charges in the molecule and its partition between lipid and aqueous phases should determine its initial absorption and subsequent translocation. Levi tested several 2,4-D compounds and formulations. The results of one test involving three alkyl esters are presented in figure 5. Probably the mobility of the ester molecules as related to their size and weight is the factor responsible for the differences shown. As a herbicide in the field, the hexyl ester is probably as effective as the methyl ester, if not more so, and the octadecyl ester will give a high degree of bending within 24 hours. The molecular weights of these esters are 234, 304, and 444, respectively. The value of this experiment lies in its demonstration that physical laws do apply to the plant functions being studied.

The next test compared several formulations of 2,4-D, namely the emulsified acid, the isopropyl ester, the propyleneglycolbutylether ester, and the butoxyethanol ester. As shown in figure 6, these molecules were all rapidly absorbed and translocated into the epicotyls of the bean plants.

A comparison of 2,4-D and 2,4,5-T acids was made using 10 μg per application and including 0.1 per cent Trem 615 as a surfactant. Figure 7, showing the results, indicates that 2,4,5-T enters the plant somewhat more slowly than 2,4-D. The relative molecular weights are 220 and 254, respectively. Theoretically, the chlorine substitutions in these molecules are lipophilic, and the third chlorine might hinder the partition of the molecule from the lipid phase of the leaf or cell surface into the aqueous medium of the living cell.

One further test was run comparing additional formulations of 2,4-D and including some 2,4,5-T formulations. Figures 7 and 8 present the results. Again the ester formulations are superior to the salts, and, in general, 2,4-D is absorbed and translocated somewhat more effectively than 2,4,5-T. It should be emphasized that the plants for these tests were grown at different dates in the greenhouse with only natural light, no control over humidity, and only rough temperature regulation. Comparisons within a test are valid, but different tests should not be compared. In the tests reported in figures 4, 5, and 7, 5 μg of 2,4-D, on the acid-equivalent basis, constituted the dose. Surfactant was included in all test solutions.

Although 2,4-D was the best translocation indicator that had been introduced into plant physiological research, it still left some questions unanswered. For instance, is it 2,4-D per se or is it a breakdown product, an association complex, or some secondary compound produced in the plant as a result of the stimulus of 2,4-D that causes bending, inhibition of leaf development, and formative effects? Is the 2,4-D translocated as the acid, the ion, or as a sugar ester when it moves with assimilates in the plant? When 2,4-D with carbon 14 in the molecule was synthesized, a more effective tool was at hand for studying both the biophysical and the biochemical aspects of 2,4-D action. With the receipt of an Atomic Energy Commission contract in June, 1951, studies using carboxyl-labeled 2,4-D were initiated, and the primary function of this paper is to report on some of these studies.

Studies Using C^{14} -labeled 2,4-D

Mode of Killing and Treatment Periods. With carboxyl-labeled 2,4-D (hereafter referred to as 2,4-D*) obtained from Tracerlab (specific activity of 1.24 mc per mM), a stock solution was prepared to give 50 μg of 2,4-D acid per 0.01 ml of solution. Because the bean-bending test had proved very erratic during the winter in the greenhouse, a bank consisting of twenty-four 8-foot slim-line tubes and sixteen 75-watt incandescent bulbs was installed to provide supplementary illumination. Fairly reproducible bending could be obtained during cloudy weather if these lights were used.

Our first experiment was exploratory. Bean plants were grown in 4-inch pots until the unifoliolate leaves were fully expanded. They were treated, usually in the morning, by placing one drop of 2,4-D* solution inside a lanolin ring at the base of the lamina of one unifoliolate leaf. In this experiment a 50- μg dosage was used, consisting of a 0.01-ml droplet of a 0.5 per cent 2,4-D* solution in 50 per cent alcohol and containing 0.1 per cent Nonic 218.

At the end of the specified treatment period the plants were removed from the pots, their roots were washed free of soil, and they were killed.

This first experiment concerned the method of killing the plants at the end of the experimental period of treatment, and the time required for autographing. Some of the plants were killed by quick-freezing between blocks of dry ice and dried between hot, dry blotters; the remainder were dried between hot, dry blotters by the method commonly used in preparing herbarium specimens. Exposure periods of the dried plants on the film were 1, 2, 4, and 8 weeks. Kodak no-screen X-ray safety film was used.

Killing of the plants started after a 4-hour period of treatment, and all plants were drying in the press within 2 hours. The dried plants were placed on the X-ray film and bundles of the films with the plants in position and separated by blotters were bound as in an ordinary plant press and placed in the dark for the requisite exposure period. The films were developed in Kodak X-ray developer and replenisher and fixed with the Kodak prepared fixer.

As for exposure period, one week produced good autographs, 2-week autographs had somewhat more detail, 4-week autographs were somewhat darker but contained no more detail, and 8-week exposures were excessive, producing very intense images that were somewhat blurred at the edges. With more experience, we have found that use of less radioactive 2,4-D in the initial treatment (5 to 10 μ g), and 4-week autographing give the best results under average conditions.

This first set of autographs had many blurs and spots that proved to result from leakage of the radiation through the blotters used to separate the films. We now separate films with separators made by cementing together two blotters with a sheet of aluminum foil between them. No further trouble with leakage has occurred.

The greatest differences shown by this experiment were between the plants killed by quick-freezing and drying compared with those killed by the slower drying procedure. The quick-killed plants gave sharp, clear autographs with the tracer well distributed throughout the petiole of the treated leaf and the stem, and, in almost every plant, present well down into the root system. In many plants it was present in the petiole and main veins of the opposite leaf. In no case was there a high concentration in the terminal bud.

In the slowly killed plants (killed between hot blotters) the concentration of tracer was less in the veins of the treated leaf and in the roots of the plants. It was not present in the petiole and veins of the opposite leaf, but it was highly concentrated in the terminal bud. These differences are illustrated in figure 9 A and B.

At the time that these experiments were conducted (January, 1952), we were at a loss to explain these differences. Now, after much work, including tests using freeze-drying, we offer the following explanation. With the dry-ice treatment all liquid in the plant is rapidly frozen. The plants come from the dry-ice treatment stiff and extremely brittle. As the tissues thaw they become extremely permeable and 2,4-D from the treated area can diffuse into the xylem and there be carried to any region where drying is taking place. This probably explains its presence in the untreated leaf and its very extensive distribution throughout the roots of the treated plants. From the standpoint of the use of radioactive 2,4-D as a tracer of phloem transport, this constitutes an artifact and gives a distorted picture that must be considered in interpreting autographs of plants killed by this method.

In the plants killed by slow drying, there is undoubtedly a considerable extension of the period of treatment during which phloem transport continues. This is shown by the high intensity of tracer in the terminal buds. On the other hand, the restricted distribution in roots, the lack of tracer in the untreated leaf, and the intensification of the image of the terminal bud all indicate that in this killing procedure restriction of transport to the phloem indicates normal movement of 2,4-D*. The undetermined extension of the treatment period is the most serious drawback to the method.

Dosage Series. A short exploratory experiment using a 2-hour treatment period, dosages of 1, 5, 10, 20, and 50 μg of radioactive 2,4-D, and killing by the quick-freezing method proved that fewer than 5 μg of tracer were insufficient under the conditions of the experiment, 10 and 20 μg gave excellent autographs, and 50 μg were about a maximum for obtaining clear autographs.

Time Series. Our next experiment was an extensive one designed to explore a wide range of treatment times. The dosage was 50 μg and the times were as follows: $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, 36, 48, 72, 96, 120, and 144 hours. The quick-killing method was used, and in interpreting the results this has to be taken into account.

The 15-minute test in this experiment showed little movement beyond the treated spot (fig. 10); the 30-minute test showed the tracer present in the roots of the plants, but its presence in the opposite leaf indicates that this is probably the result of xylem movement after killing (fig. 11). Day (1952) found that absorption and movement into the phloem required about an hour and that seems to be true in this case also (fig. 12). In the 2-hour test, four out of five plants had 2,4-D* in the roots; in the 3-hour test, five out of five had 2,4-D* in the roots and four of the plants were bent (fig. 13); however, in 4- and 5-hour tests, one plant in each lot failed to translocate 2,4-D* into the roots. Beyond the 5-hour treatment period all plants had 2,4-D* in their roots, and beginning with the 6-hour period, 2,4-D* was present in the terminal buds, increasing in concentration with increasing treatment time (fig. 14). Since all these plants were quick-killed, they should give a critical measure of the time required for movement into this meristematic tissue. Apparently in the first experiment (killed by drying between blotters) the large difference in intensity of the images of the terminal buds represented movement into the bud after the plants were removed from the soil and placed in the press. The image intensity of the terminal buds in the plants dried without quick killing corresponded with that of the quick-killed plants of the time experiment having a treatment period of 24 hours.

In this treatment-time experiment, bending of the epicotyls was evident in two out of five plants treated for 2 hours; in four out of five treated 3 hours, and to varying degrees in nearly all plants treated for longer periods. Wherever there was bending, 2,4-D* was present in the epicotyl.

An outstanding result in this time series was the gradual loss of intensity in the roots, treated leaves, and opposite leaves after 24 hours. This probably represents actual loss of the C^{14} in the form of C^{14}O_2 from the plants as found by Weintraub *et al.* (1952). Figures 15, 16, and 17 show autographs of some of the plants described. Figure 18, *A* and *B*, shows an effect noted in the $\frac{1}{2}$ -hour and 1-hour tests. Our interpretation of this phenomenon is that there was leakage through the cuticle directly into the

epidermis and mesophyll cells and thence into the xylem, and distribution to the leaf tip in the transpiration stream. After 1 hour, such 2,4-D* should be picked up and moved out via the phloem. The fact that this one-way movement is not shown in any of the longer treatment times seems to justify this view.

Soil Moisture. In his preliminary studies on the "bean test," Day (1950, 1952) found that it was necessary to water his plants fairly soon before treating them because they would not bend if the soil moisture was approaching the permanent wilting percentage. To find out if this was caused by lack of absorption and translocation or just by lack of the bending response, an experiment was set up to determine the relation of soil moisture to the movement of 2,4-D*. In this experiment plants grown as usual were watered 48, 24, and 3 hours before treatment. The dosage was 50 μg of 2,4-D* with Nonic 218; treatment time was 2 hours. The roots were cleaned as well as possible without washing. As the plants were harvested they were cut into (1) treated leaf plus petiole, (2) untreated leaf plus petiole, (3) epicotyl and hypocotyl, and (4) roots. The fractions were quick-frozen and dried in the usual fashion. At time of harvest the plants watered 3 hours previously were bent; the other two groups had straight epicotyls.

In studying the autographs of this experiment, three features were prominent: (1) in no case was there any 2,4-D* in the opposite leaf or in the roots; (2) 2,4-D* was present in the stems of straight as well as bent plants; (3) translocation of 2,4-D* into the hypocotyls of the plants having ample soil moisture was more prominent than in those from which water had been withheld, but the differences were small. Evidently dissection of the plants had eliminated the artifact of xylem transport experienced in the other tests.

A second experiment on the role of plant-water relations in 2,4-D* transport involved three sets of plants. All plants were grown as usual except that water was withheld for 24 hours before treatment. In this experiment the dosage was 50 μg of 2,4-D* with 0.1 per cent Nonic 218; treatment period was 4 hours. Immediately after application of the 2,4-D* five plants were placed in a humid chamber with a glass top, five were left under the bank of lights in the open greenhouse, and five were placed outside on the south side of the greenhouse in the bright sun. This latter constituted a low-humidity treatment. These plants were harvested whole, quick-frozen, and dried by the usual method. At the time of harvest the plants from the humid chamber were bent, one of five from the greenhouse bench was slightly bent, and the five from out-of-doors were straight. Figure 19, A, B, and C, shows the autograph of one plant from each environment. These were chosen to illustrate the average condition. All plants had 2,4-D* in the untreated leaves and in the roots. However, those from the outside had the greatest intensity of 2,4-D* in the epicotyl and hypocotyl; those under greenhouse conditions and those from the humid chamber slightly less. The 2,4-D* had moved into the epicotyls and hypocotyls of unbent as well as bent plants.

Thus, in two separate experiments bending was shown to be a function of the water balance of the plant as well as an indication of the presence of 2,4-D*. Because this last experiment had a treatment time of 4 hours, presence of 2,4-D* in the roots probably represented phloem transport; as in the stems, the amount in the roots was less in the plants under dry conditions, but

the differences were not great. The velocity of movement was apparently the same in all three conditions, but the amounts moved depended upon environmental conditions. One factor of importance here was the rate of drying; plants in the moist chamber retained the 2,4-D* in solution for much longer periods than did those in the open air. Figure 19, A, showing the high intensity of radioactivity in the apical portion of the treated leaf, reflects this favorable condition for prolonged absorption.

Placement Series. The next experiment concerned the placement of the droplet in treating the bean plant. Day and Levi, in earlier tests, had shown that treating young, rapidly expanding leaves resulted in little or no bending. And Day (1950) showed that placing the droplet over the main veins at the base of the lamina gave the most pronounced bending response. Treating the underside of the leaf was more effective than treating the upper surface, but this imposes a practical hardship; since treatment on the upper surface gives satisfactory response, this method has been made standard practice.

McCarthy performed two experiments on placement of the droplet on the leaf or bud and one on application to roots. In the leaf treatments, droplets were placed (1) at the base of the lamina over the main veins, (2) at the same place on both leaves of each plant, (3) in the center of the leaf over the midrib, (4) over the midrib at the apex of the leaf, (5) on one spot at the edge of the leaf, and (6) on five spots around the periphery of the leaf. There was also a treatment in which application was made on the terminal bud. Treatment time was 4 hours; some treatments were at 50 μ g, others at 20 μ g. Killing was by quick-freezing.

Plants treated through the roots were grown in Hoagland's solution until ready for the experiment. They were then removed; their roots were rinsed in distilled water and placed in a 1 ppm solution of 2,4-D* for periods varying from $\frac{1}{2}$ hour to 8 hours. They were removed, rinsed twice in distilled water, quick-frozen, dried, and autographed.

Plants treated at the base of one or both leaves showed the same pattern of distribution of radioactivity, namely, movement throughout the stem and well into the roots. Intensity in the stems was high (fig. 20 A and B). Treatment over the midrib in the center of the leaf was equally effective during the 4-hour treatment period (fig. 21). Treatment at the apex of the leaf, on one basal lobe, or at five different positions around the periphery resulted in about the same distribution into the stem and roots, but in all cases the intensity of the autographs was materially reduced (figs. 22 A, B; 23).

Plants with their roots in 1 ppm 2,4-D* for 4 and 8 hours had the tracer throughout the roots, and some, but not all, had it up the stems and into the leaves. Evidently this compound is not rapidly absorbed and concentrated in bean plants, and more concentrated bathing solutions will be needed to give good autographs. The problem is to get absorption from solutions that will not cause serious injury of root cells. This was accomplished in later tests.

Experiments involving the presence or absence of wetting agent proved that these additives enhance 2,4-D* absorption and give more intense autographs, other factors being the same. Distribution of the tracer was not changed. Other wetting-agent tests showed that concentrations of 1.0 and 0.1 per cent made no difference in concentration or distribution of absorbed 2,4-D*.

Transport from Different Leaves. One experiment with bean plants attempted a study of transport from different leaves. The plants were allowed to grow until the two unifoliolate leaves and one trifoliolate leaf were fully expanded. Applications were of $5\text{ }\mu\text{g}$ of 2,4-D* in 50 per cent alcohol with 0.1 per cent Nonic 218. The treatment period was 2 hours. The plants were quick-frozen and dried between blotters.

Treatments on the terminal buds and on young trifoliolate leaves resulted in no movement from the treated regions. On the second trifoliolate leaf, treatment resulted in movement only within the leaf. On the first trifoliolate leaf, treatment resulted in movement into the roots in low concentration; on unifoliolate leaves, movement into roots was more prominent; the tracer also moved out into the young stems and leaves at the tips of the plants.

Several attempts to check on movement during thawing and drying, by fractionating the plants in the frozen state, indicate less absorption when the plant is so fractionated, but loss of sap from the thawed plant parts when they are pressed between blotters obscures the results.

Killing and Drying Methods. A number of experiments have been conducted attempting to evaluate the artifact of xylem transport during drying of quick-frozen plants. In these, freeze-dried plants have been compared with plants dried between warm blotters after quick-freezing. McCarthy performed one such experiment, and though he was not certain that the plants had not thawed during drying, through inadequacy of the freeze-drying equipment, he did find that much less 2,4-D* was present in the freeze-dried plants. The treatment period was 4 hours, and distribution was about the same in both sets of plants.

Later freeze-dry experiments⁷ confirm the finding that less 2,4-D* is moved when the plants are dried in the frozen condition. The first freeze-drying experiment compared four freeze-dried plants with four that were cut into leaves, petioles, stem, and roots while frozen and subsequently dried as usual, and four that were quick-frozen and dried between warm blotters. All plants were treated for 1 hour with $5\text{ }\mu\text{g}$ of 2,4-D*. The fractionated plants were placed between sheets of filter paper, and the spots on these sheets were autographed. The freeze-dried plants were treated for 1 hour, quick-frozen, placed between sheets of $\frac{1}{8}$ -inch mesh hardware cloth, and immediately covered with pulverized dry ice. Four plants were stacked and placed within a precooled steel vacuum chamber containing calcium hydride, and the chamber was then placed within the freezing unit of a domestic refrigerator. A vacuum pump attached to the vacuum chamber was started and allowed to run throughout the drying period of 5 days. A thermocouple inside the vacuum chamber was used to check on the temperature, which did not rise above -7°C during the 5-day period. The vacuum maintained after the dry ice had sublimed averaged around 3 mm of mercury. The plants came out of the chamber in a very dry condition.

Autographing revealed the intensity in the stems to be very low, and while the roots could be seen on the autographs they were at the lower limit of visibility. The fractionated plants had even less intensity in the stems, but autographing of the filter papers between which they were dried showed appreciable leakage from the cut ends. The quick-frozen, blotter-dried plants had more 2,4-D* throughout their stems and roots. Considering that the

⁷ Conducted by James E. Pallas, Jr.

treatment lasted only 1 hour, the period of time that Day (1952) had found necessary for absorption by the leaf, these results are to be expected. They prove that the higher concentrations of tracer found in blotter-dried plants, and the very rapid distribution found in certain experiments (Crafts, 1953a, also fig. 12), are the result of the killing process, or, more specifically, the thawing and drying following quick-freezing. This places a definite limitation on the interpretation of autographs of plants killed by such methods. Later experiments with treatments lasting as long as 24 hours show that 2,4-D* is present in the roots in 4-hour-treated plants, and in 24-hour treatments the roots autograph very strongly as shown in figures 25 A, B, and 26.

Cotton Plants. Because cotton is highly sensitive to 2,4-D and gives pronounced formative effects, it was used for translocation studies by Clor (1951) as reported above. In order to check on Clor's results, McCarthy performed three experiments on young cotton plants, using the labeled 2,4-D*.

The first used plants 2, 3, 4, and 7 inches high; treatment was with 10 μ g in some instances and 50 in others, the droplets being placed on one cotyledon in each case. The treatment period was 4 hours and killing was by quick-freezing. Results were as follows: (1) in small plants (2 inches) with cotyledons still expanding, outward movement was slight; (2) with larger plants with cotyledons fully expanded and green there was strong movement into the stem and root (fig. 27); (3) with plants somewhat larger and the true leaves starting to expand, transport was strong and some 2,4-D* moved into the expanding leaves (fig. 28); (4) in the case of 7-inch plants with four leaves expanded above the cotyledons, translocation from a cotyledon was outward and both downward to roots and upward into expanding leaves (fig. 29).

In the next experiment, where treatment was on the second and fourth expanded leaves, movement from the second leaf was both downward and upward (fig. 30), whereas movement from the fourth leaf, which was still expanding, was very slight in the downward direction (fig. 31).

In a third experiment, where the treatment time was 2½ days, movement from the cotyledon of 4-inch plants was both downward into the roots and upward into expanding leaves. When application was upon the second expanded leaf, it was both downward into the hypocotyl and roots and upward into young expanding leaves.

In general, the results confirm Clor's studies (1951) indicating that translocation of 2,4-D* accompanies assimilates in their movement from regions of synthesis to regions of utilization.

Cucumber Plants. For further confirmation of this translocation pattern a parallel experiment was run with cucumber seedlings.* Seeds were started in sand and, after one week, transferred to full-strength Hoagland's solution in aerated culture tanks. Growth was rapid, and at 10 days the cotyledons were fully expanded and green; at 14 days the first true leaf was expanded; at 17 days the second true leaf was expanded and the plants were of correct size to autograph on 10 × 12-inch film. Applications were made in four locations: cotyledon, first leaf, second leaf, and third leaf. Treatment times were ¼, ½, 1, 2, and 4 hours. Dosage was 5 μ g of 2,4-D* with a specific activity of 1.24 mc per mM. The 2,4-D* was dissolved in 50 per cent alcohol at a concentration of 500 ppm, and the solution contained

* Conducted by James E. Pallas, Jr.

0.1 per cent Nonic 218. A lanolin ring was used to confine the droplet. At the end of the treatment time each plant was removed from the culture solution, quick-frozen, and dried between blotters.

Movement in these plants was rapid. Although the possible artifact of xylem movement has to be taken into account in the $\frac{1}{4}$ - and $\frac{1}{2}$ -hour treatments, in the 1-, 2-, and 4-hour periods movement into the roots from the cotyledon and first leaf was prominent, from the second leaf it was much less, and from the small third leaf it was very slight. When movement from the young third leaf was prominent, the petiole and adjacent stem were very dense, indicating possible spread of the treating solution from the leaf onto the stem. The lanolin ring used to confine the solution is difficult to make on such young leaves as they are still folded and very hairy.

All treatments were made in duplicate and figures 32, 33, 34, and 35 show one of each of the 1-hour treatments. It is very evident that translocation into the roots is much more prominent from the cotyledon and first-leaf treatments. This result is borne out by the other autographs of this experiment and serves to indicate again that 2,4-D* moves with assimilates in this species and not along an independent gradient.

DISCUSSION AND CONCLUSIONS

The foregoing presentation of experimental results of studies with radioactive 2,4-D as a translocation tracer emphasizes the difficulties involved in this type of research. Despite the many virtues of this material as a tracer, unless the experiments are carefully designed and correctly interpreted, one can be led into false conclusions. Probably the best way to indicate the difficulties is to analyze carefully the many possible reactions that may result from the simple procedure of placing a droplet of the tracer solution on a leaf of the test plant.

Colwell (1942) showed that when a large area of a squash leaf is wet with an aqueous solution of radioactive phosphorus, the tracer solution is drawn into the leaf and down through the petiole and stem via the xylem. To avoid this, he had to limit the size of area treated so that the water in the applied solution was evaporated from the treated leaf. Under these conditions, the movement of the tracer out of the leaf was confined to the phloem as indicated by its failure to move through a steamed petiole. Bidulph and Markle (1944) showed that even though the whole vein including the xylem was cut to introduce radiophosphorus into the cotton leaf, by limiting the absorption time of the test solution to 5 minutes, outward transport was limited to the phloem. Here, the method of cutting the vein so that initial movement of the test solution was toward the periphery of the leaf, and the time limitation in absorption were so controlled that back-flow through the xylem was prevented. Carrying the analysis further, if a test solution is applied to a leaf with intact cuticle having no stomates in the treated area, absorption through the cuticle should confine the tracer to the fat-like cuticle and such cell phases as could receive it by partition. Theoretically, such a tracer could be confined to living cells of the epidermis and mesophyll and could move via these cells to the phloem for transport from the leaf. In short, uptake and transport would be confined to the symplast (Münch, 1930).

When one considers that evaporation of water from the leaf by trans-

piration takes place from moist cell walls of the mesophyll, and further, that the continuous-wall phase, the apoplast, is a hydrated colloidal medium in dynamic equilibrium through imbibitional forces with water in the xylem, it is evident that any source of water at atmospheric pressure that comes into physical contact with the apoplast at any point will serve as a source for uptake and flow. Hence, any aperture in the cuticle layer, or any open stomates, or even any cuticle surface that is not completely isolated from the hydrated apoplast may serve as an opening through which liquid may reach the xylem. However, this phenomenon is probably relatively unimportant except where there is severe injury to the leaf such as occurs following quick-freezing. While open stomates are not ports of entry for aqueous solutions under normal conditions, when the test solution being applied to a leaf contains alcohol and a surfactant, as was the case in many of the solutions used in the experiments herein described, entry may be effected, and once the tracer is inside the stomatal chamber it can reach the xylem and move in it. Lambertz (1954) has recently shown that the cuticle of many species is pierced by innumerable plasmodesmata. These, too, could serve as ports of entry for chemicals.

The upper surface of the bean leaf has stomates and so, with this plant, the cuticle cannot bring about isolation of a test solution containing surfactant. However, an application of only 0.01 ml of solution, which often dries within 15 minutes, would not seem enough to allow backflow from the leaf via the xylem. That this is true is shown by the pH studies (table 1, fig. 2), which indicate that 2,4-D enters the plant across a lipid barrier—the intact cuticle of cutinized or suberized mesophyll walls (Scott, 1948). If this is true, the tracer, to move as it does, must be taken up by living cells and moved out of the leaf via the phloem. The many evidences for movement with assimilates (see review in Crafts, 1952) strengthen this view.

The above conclusions apply to intact living plants as used in the bean-curvature test. Proceeding now to tests with the radioactive tracer, when carbon 14 is used, as it was in the 2,4-D* used in the present work, detection or measurement of the radiation requires extraction and counting, or autographing of the plants. Both of these processes require killing the plants and this presents difficulties. In normal greenhouse plants the xylem operates under a greatly reduced pressure, and the phloem under a fairly high positive pressure. Therefore, when a plant is cut, movement within these systems is extremely rapid (Kennedy and Crafts, 1930; Crafts, 1936), and the fractions of the cut plant do not represent the state of the intact one. For this reason, any technique involving cutting or grinding is objectionable. Additional objections to the counting method, as far as this problem is concerned, are the difficulties with extraction and the laboriousness of preparation. Counting was actually used in our studies to check concentrations found in autographs, to analyze extracts that were chromatographed to identify 2,4-D, and to calibrate test solutions. For the bulk of our work, however, for the reasons noted above, autographing was used.

In order to detect and measure the radioactive tracer in a plant by the autographing method, it is necessary to kill and dry the plant so that it may be placed on X-ray film to give the exposure necessary. We used drying between warm blotters, quick-freezing with dry ice, followed by drying between blotters for our initial studies. The objection to the first method is

the time required for killing the plant and stopping all physiological processes. Our tests indicated that the heating probably speeded up the processes of translocation until drying of the tissues finally stopped them. This means that the treatment time was prolonged for an unknown period.

The quick-freezing method gave excellent, sharp autographs and a number of experiments were completed (Crafts, 1953a) before it was fully appreciated that transport was considerably more extensive in these plants than in plants in which all activity was stopped immediately (freeze-dried plants). A careful analysis explains why this is so. When the plant is quick-frozen, all moisture in the tissues is solidified and the plant comes from the treatment stiff and rigid. Soon it thaws, and after a few minutes between filter papers backed by warm blotters it is perfectly flaccid. Pressing will then remove considerable quantities of sap from the tissues. In the case of 2,4-D*-treated plants, this sap is contaminated and will produce an autograph. Figure 36 shows a normal autograph and figure 37 shows the autograph produced by placing the filter paper backing upon a film for four weeks.

Freezing, in this case, kills the cells of the plant and renders them completely permeable. As the plant thaws, solution moves through the tissues to satisfy gradients established by drying. And apparently 2,4-D* in solution in the sap of mesophyll of treated leaves may move into the open xylem tubes and translocate rapidly to the tip of the treated leaf, down the petiole to the stem and root, and via the anastomosed bundles of the nodes to the opposite leaf. Careful study suggests that a strong image of the veins of the opposite (untreated) leaf of bean indicates such xylem transport. A light, blurred image may result from 2,4-D* carried in through the phloem when conditions are right for import of assimilates. Movement from the node subtending the unifoliolate leaves upward into the bud or young expanding shoot is also phloem-limited, at least while this young shoot is only about 1 cm in length.

From this analysis it is evident that with the killing of the cells the symplast is destroyed as an osmotic system and the apoplast becomes continuous throughout the plant. Liquid within cells, or within tissues such as the phloem and xylem, becomes free to follow hydrostatic gradients not related to the previous functioning of the living plant but related only to the drying process. Distribution of a tracer by this system therefore becomes an artifact that may not be directly related to normal transport and hence is unreliable as an index of normal function. How serious is this as an error in the experiments previously reported (Crafts, 1953a)?

Obviously, absorption through the leaf surface, migration across the mesophyll, and transport through the phloem are three distinct processes, each requiring a definite time. According to Day (1950, 1952), absorption and migration to the phloem require about 60 minutes. Careful study of many autographs made in the studies being reported indicates that from 1 to 2 hours were required in these tests. Translocation within the phloem is known to be quite rapid (Crafts, 1951; Biddulph, 1954). Day found an average velocity of about 50 cm per hour. This would indicate that it takes about 10 to 15 minutes for 2,4-D* to move from the treated leaf to the epicotyl and two to three times as long for it to move throughout the roots. From this it seems evident that the movement indicated in figures 6 and 8 of

an earlier paper (Crafts, 1953a) was brought about by xylem transport during drying and not by phloem transport during the 30-minute treatment period. The presence of 2,4-D* in the petiole of the opposite leaf is evidence for this same effect. While the autographs in figures 9 to 13 may also contain this artifact, it evidently is not responsible for the differences shown because all plants were killed by the same method. Careful studies of several hundred autographs indicate that, with the exception of the untreated leaf, distribution of 2,4-D* in plants given treatment periods of 2 hours or more is accurately shown in autographs. It is in the short-time experiments that the artifact is prominent, and autographs of plants in these experiments should be viewed with this in mind. In this connection it should be pointed out that the test involving the plant shown in figures 6 and 8 of the 1953 paper included five plants only two of which showed such extensive movement. In the other three the 2,4-D* had reached only into the petioles. If Day (1950, 1952) was correct in concluding that absorption and migration to the phloem take about 60 minutes, and this conclusion was based on well replicated tests, then even these other three plants give an erroneous picture. The problem here is plant variability, and the most certain thing about these tests is that the plants are quite variable. Plants dried in the frozen condition should give a true picture of phloem transport of the tracer. As shown by figures 25 and 26, transport into the roots of bean plants occurs in 4 hours, and distribution is complete in 24 hours.

Turning now to the tests involving longer treatment periods, it seems evident that these studies with 2,4-D* substantiate the view that this chemical can penetrate leaf tissues and move readily with assimilates in the phloem. Several practical applications can be made of these findings. First, with regard to penetration, the studies on pH and the tests with esters and with surfactants all show that where translocation of the 2,4-D is desirable, formulation is of extreme importance. The pII studies show that somewhere in the leaf a lipid barrier must be passed, and here the fat solubility of the acid and esters has a bearing. The comparative test on esters points out the influence of chain length on mobility in the lipid phase and on partition characteristics on the aqueous side. And the surfactant studies show the benefits of reducing the interfacial tension at the leaf surface to allow very intimate contact of the herbicide molecules with the cuticle. Since adsorption and penetration of the 2,4-D depend on this intimate contact, a suitable surfactant is a necessary constituent of a 2,4-D formulation that is designed for penetration and translocation (Orgell, 1954). One gains the impression, in talking to salesmen and company representatives, that the inclusion of surfactants has been mainly aimed at low cost and convenient emulsion stability. These studies indicate a much more important function and one wonders if this has been kept in view in testing surfactants for 2,4-D formulation.

When the differences in penetration and movement of different esters are studied, one wonders again if these factors have been sufficiently considered in testing esters. This is particularly pertinent with respect to the heavy esters now being formulated. Hundreds of esters and hundreds of surfactants could be and actually are being synthesized.

Some of the newer emulsifiable acid and heavy ester formulations are killing weed species that simply would not respond to the old salt and

alkyl ester products. This proves the importance of formulation and emphasizes the role of balanced solubility, lowered interfacial tension, and proper molecular configuration in herbicidal function. And after the ideal mixtures for the common weed species have been found, there remains a host of special problems relating, for example, to odd tree and brush species, to riparian vegetation, to some of the legume species, such as aruba, broom, and gorse, to fern and cycad species in the tropics. After more than 100 years of agricultural research we are still investigating the use of simple chemicals like nitrate fertilizers. Undoubtedly it will take as long or longer to work out all of the details in the most effective use of 2,4-D. And as the studies reported herein show, the use of radioactive isotopes as tools in this research will not solve these problems overnight. Although these tools are time-saving and convenient, they must be used with caution, and with a full realization of all possible sources of error.

In addition to formulation problems, there remain those relating to the physiology of the treated plants. And in the case of selective action, the physiology of both weed and crop species must be considered.

From the studies on cotton and cucumber plants it is evident that applied 2,4-D may be transported to the roots or to the shoot, it may move to both root and shoot, and under certain conditions it may not leave the treated leaf. When the whole plant is sprayed, the problem is somewhat simplified. Will the bulk of the applied chemical go to the roots, will it go to the shoots, or will it stay in or on the treated leaf? Obviously, this is important in the control of perennial weeds.

In considering the movement of assimilates in perennial weeds, it has been found that, in the early spring, carbohydrate reserves in the roots move up to provide for the nutrition of the growing shoots (Robbins, Crafts, and Raynor, 1952). Spraying young foliage with 2,4-D has proved completely ineffective in killing the roots. About the time that shoot buds are forming on many perennials, the assimilate stream moves from the foliage to the roots, and the roots are still actively growing. At this time and until early blossoming, spraying is usually very effective in producing root kill. During late blossoming and seed maturation the assimilate stream moves to the roots, but these are mature and apical growth often has ceased. Translocation of 2,4-D may be effective, but the mature root tissues are not killed. This tells us why 2,4-D treatments must be properly timed, and the work with radioactive 2,4-D has clarified the picture.

Concerning application methods, the experiments illustrated in figures 20, 21, 22, and 23 show that the actual size and location of spray droplets can be important. This is reflected in the common recommendation that sprays be applied to weeds in the form of coarse droplets; particularly is this true in treating brush (Fisher, 1952). Coarse-droplet application is essential only in application of translocated herbicides. This contrasts with the recommended method for applying contact herbicides and certain insecticides where complete coverage is essential.

Concerning the mechanism involved in the movement of 2,4-D in the phloem, recent proof that the sieve tubes in this tissue retain the property of semipermeability and plasmolizability (Currier, Esau, and Cheadle, 1955) indicates the very special properties that must be attributed to this herbicide. Apparently 2,4-D and similar translocated compounds move

through these highly specialized cells in concentrations that kill roots without rapidly injuring the living conduits. Eventually there may be phloem necrosis (Eames, 1950), but during the hours when translocation is taking place, injury is not sufficient to prevent functioning of the phloem system.

This is a further indication that dosage must be regulated to minimize contact injury where translocation is essential to successful use of the method.

SUMMARY

Translocation of an herbicide is essential if the roots of a plant are to be killed by foliage spraying. Early work indicates that 2,4-D permeates the cuticle of sprayed leaves, migrates to the phloem, and is transported in this tissue, along with food materials in the plant. This paper reports work with labeled 2,4-D, and tends to substantiate the early conclusions.

When radioactive 2,4-D (2,4-D*) is used as a tracer, the methods of killing and drying the plants for autographing are critical. Freeze-drying proved to be the most reliable method. In bean plants, 2,4-D* was absorbed and translocated into the roots in about three hours. Bending of the epicotyls took two to three hours, and 2,4-D* was present in all bent epicotyls. Movement into terminal buds took about six hours. After 24 hours, intensity of 2,4-D* in autographs decreased in roots, treated leaves, and opposite leaves. This probably represents metabolism of the chemical and loss in the form of CO₂.

Translocation of 2,4-D* took place in plants that did not bend because of water deficiency. Placement of the 2,4-D* on the leaf determined the relative amount of chemical moved; over the midrib at the base of the leaf was the most favorable position. Absorption of 2,4-D* was enhanced by the presence of a surfactant in the applied droplet. In many-leafed bean plants, absorption and translocation were greatest from the lower unifoliate leaves, less from successively higher leaves.

Translocation of 2,4-D* into stems and roots of cotton and cucumber plants was greatest from cotyledons, less from successively higher leaves. No 2,4-D* was transported from young leaves that were still importing foods from more mature parts of the plants.

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Fig. 1. Effect of 2,4-D on cotton. Symptoms (curling) on the leaves of the untreated plant (left). The plant to the right was treated with 16 μ g of 2,4-D.

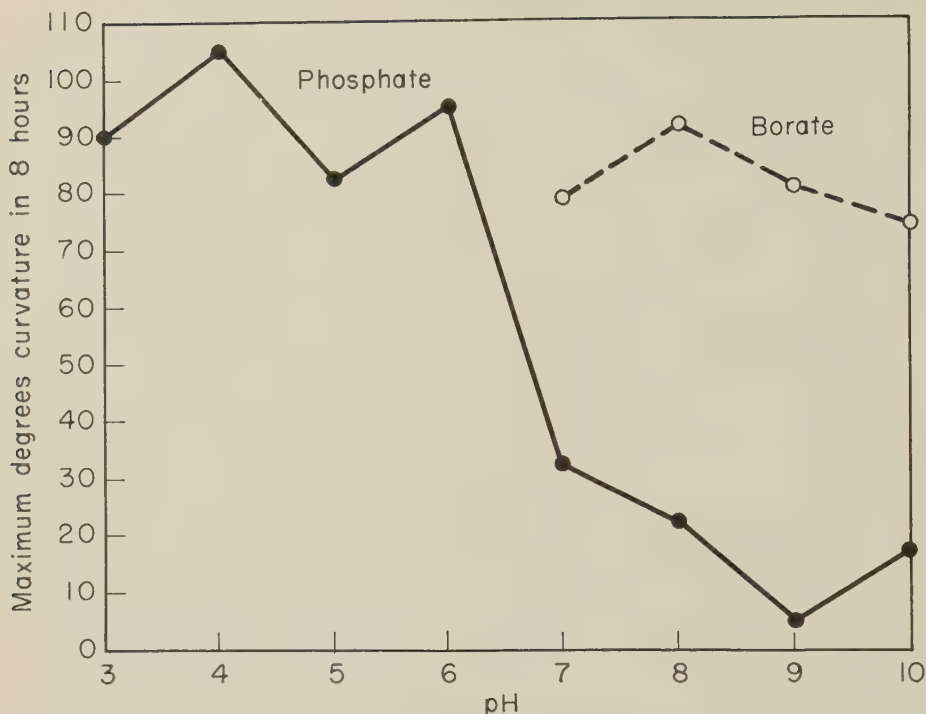


Fig. 2. Comparison of curvatures at high pH with 2,4-D solutions buffered with phosphate and with borate. Black-eyed peas, 2 replicates for the phosphate and 8 for the borate. Wetting agent, Trem 615, 0.1 per cent; 1.0 μ g 2,4-D.

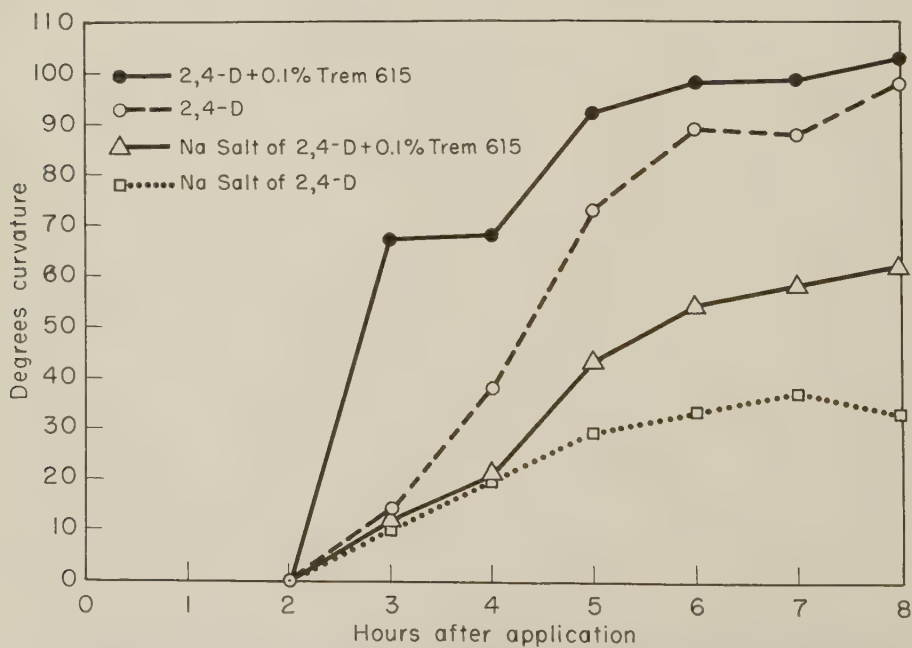


Fig. 3. Comparison of curvatures of bean plants from 2,4-D acid and its sodium salt, with and without wetting agent. Five replicates; 10 μ g of acid equivalent per leaf.

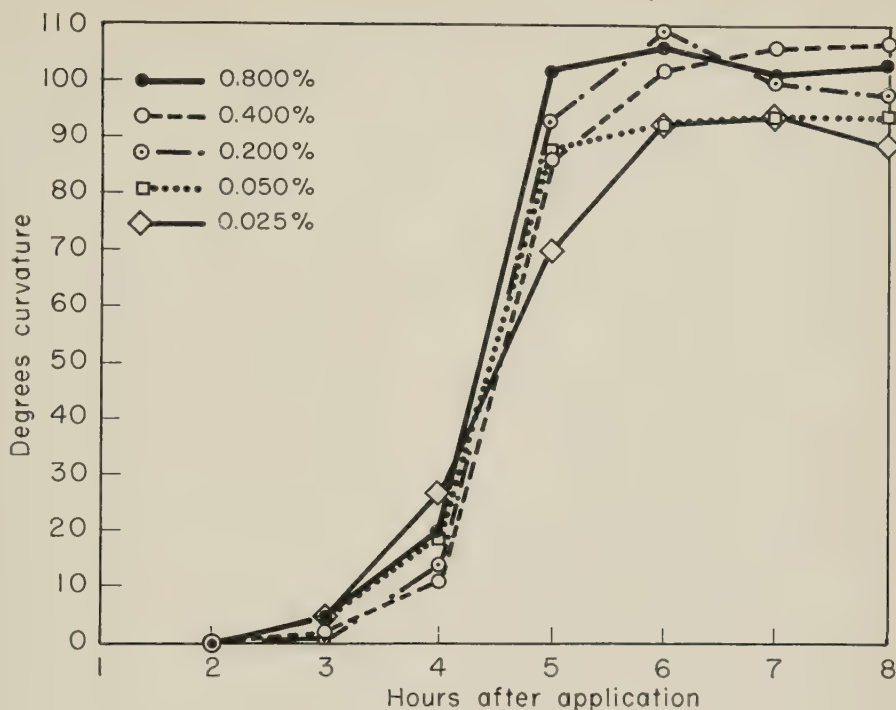


Fig. 4. Comparison of curvature at 10.0 μ g of 2,4-D with different concentrations of wetting agent, Trem 615. Beans, 5 replicates.

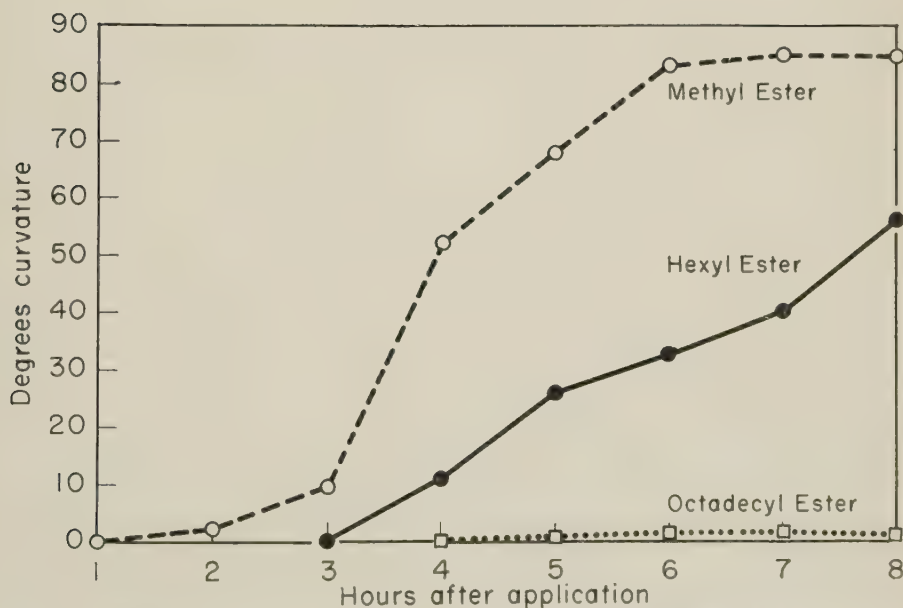


Fig. 5. Comparison of curvatures with three alkyl esters of 2,4-D. Beans, 5 replicates; 5.0 μ g of acid equivalent; wetting agent, Mersox (Monsanto Chemical Co.), 0.1 per cent.

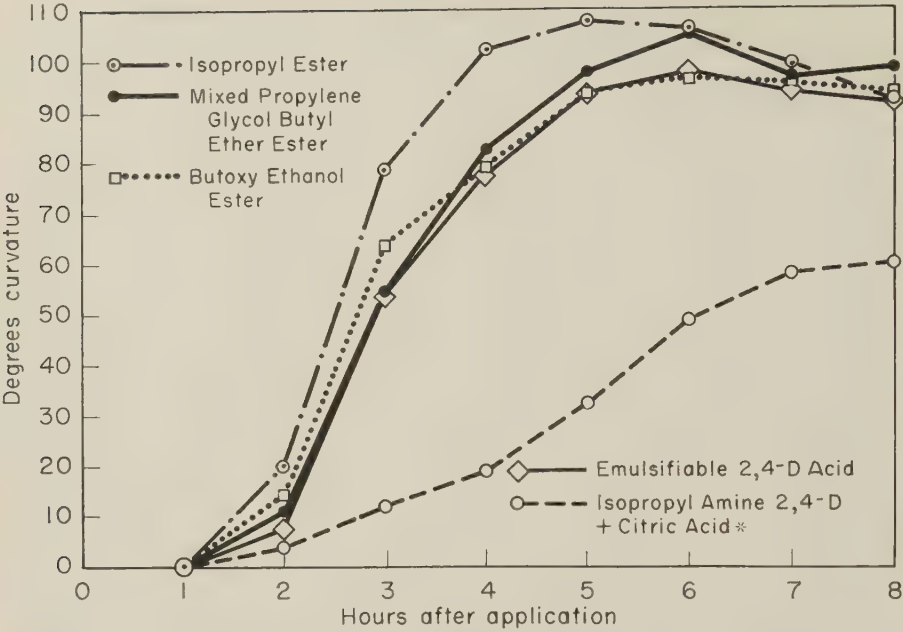


Fig. 6. Comparison of different formulations of 2,4-D by bean curvature. Beans, 10 replicates; 5.0 μ g of acid equivalent. Formulations originally contained wetting agent. * was not formulated and 0.1 per cent Trem 615 was added.

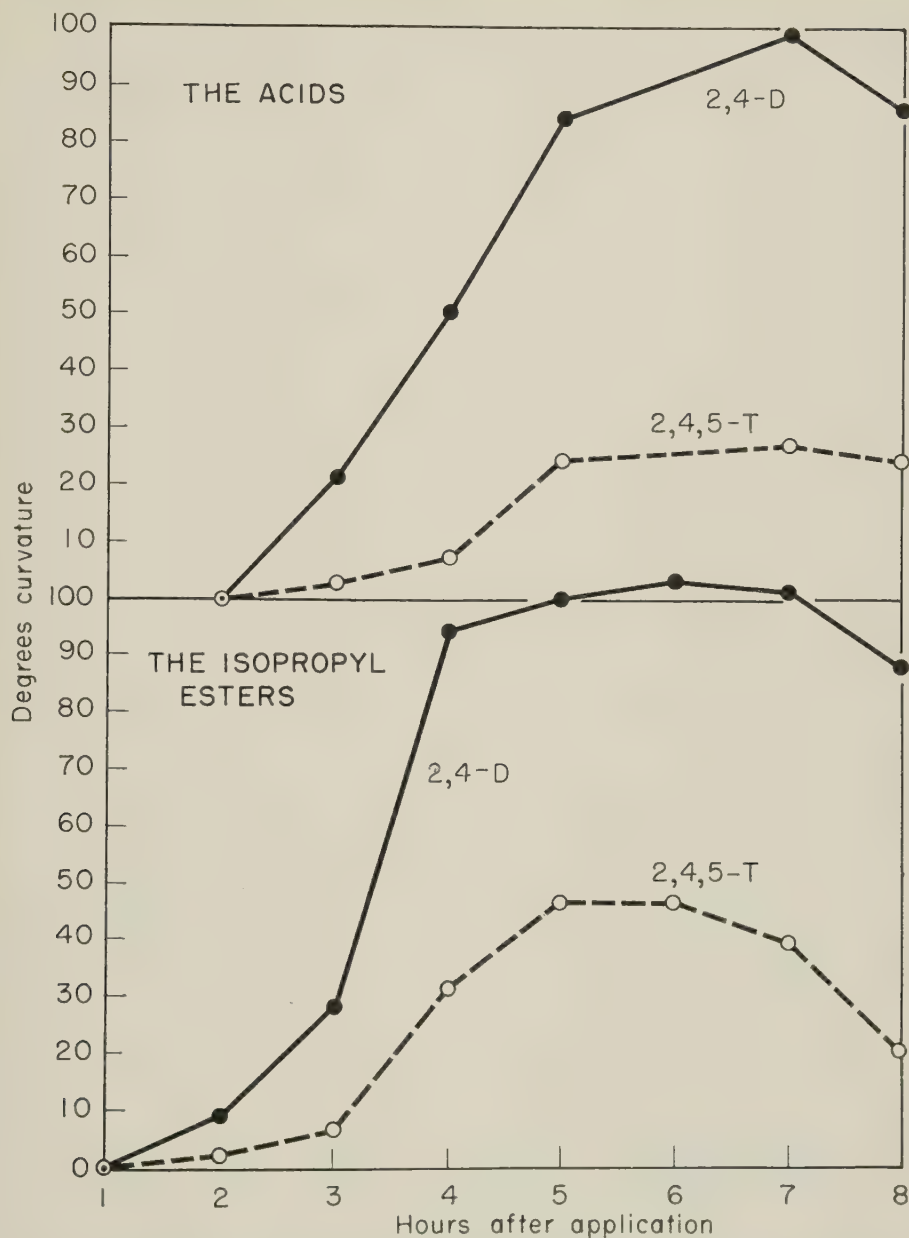


Fig. 7. Comparison of corresponding compounds of 2,4-D and 2,4,5-T using bean curvature. Beans, 5 replicates; 5.0 μg of acid equivalent. Formulations originally contained wetting agent.

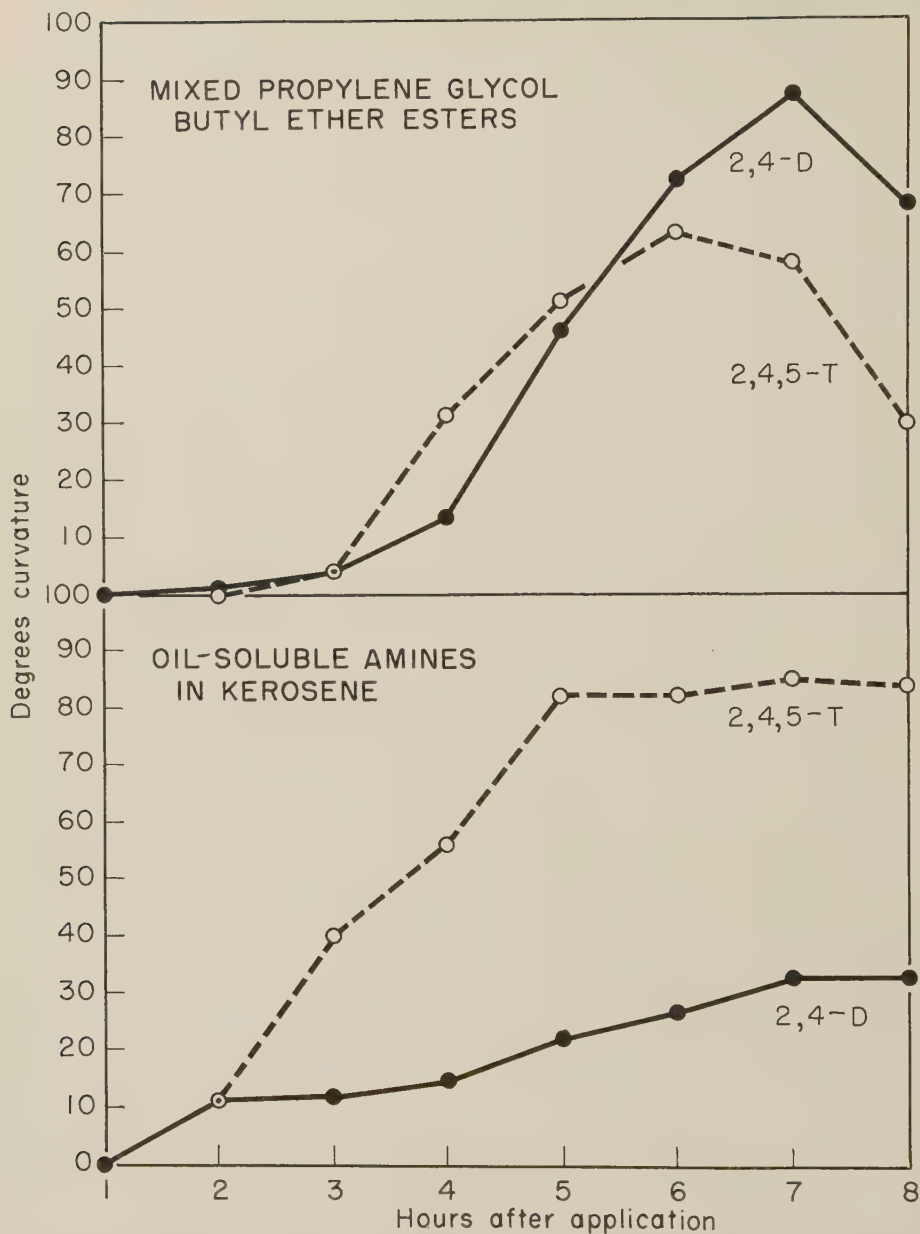


Fig. 8. Comparison of curvatures with corresponding compounds of 2,4-D and 2,4,5-T. Beans, 5 replicates. 5.0 μ g of acid equivalent. Formulations originally contained wetting agent.



Fig. 9. *A*, radioautograph of a bean plant killed by drying between hot, dry blotters. Dosage 50 μ g of 2,4-D*, treatment 4 hours, exposure 4 weeks. *B*, radioautograph of a bean plant killed between blocks of dry ice and dried between blotters. Dosage 50 μ g, treatment 4 hours, exposure 4 weeks.



Fig. 10. Radioautograph of plant killed by quick-freezing and blotter-drying. Dosage 50 μ g, treatment 15 minutes, exposure 4 weeks. Left, ozalid print of the dried plant; right, radioautograph.

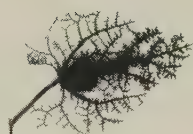


Fig. 11. A 30-minute treatment; other conditions as in figure 10.

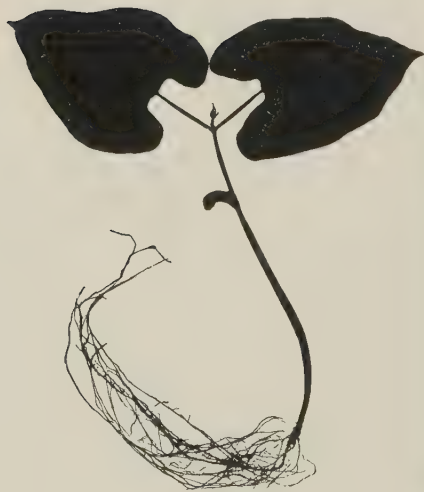


Fig. 12. Radioautograph of plant with 1-hour treatment period.



Fig. 13. Treatment period 3 hours.



Fig. 14. Treatment period 8 hours.

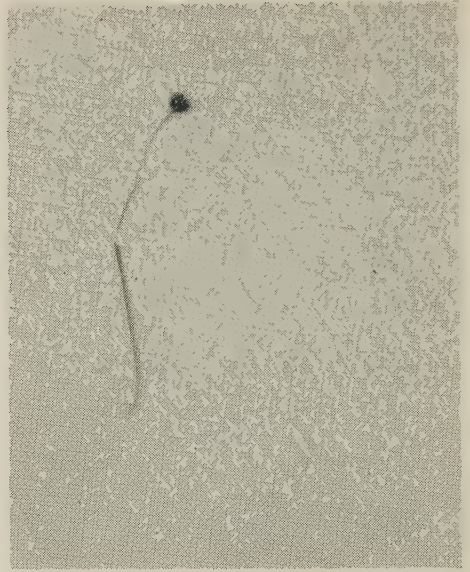


Fig. 15. Treatment period 24 hours.



Fig. 16. Treatment period 72 hours.



Fig. 17. Treatment period 144 hours.

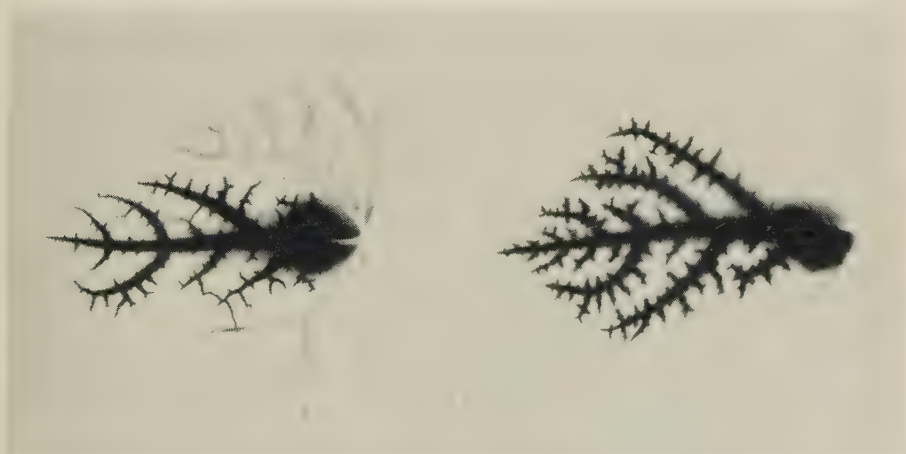


Fig. 18. Radioautographs of bean leaves of plants with dosage 50 μ g, treatment period 30 minutes, exposure 4 weeks. Movement here has been only in the acropetal direction in the treated leaves.

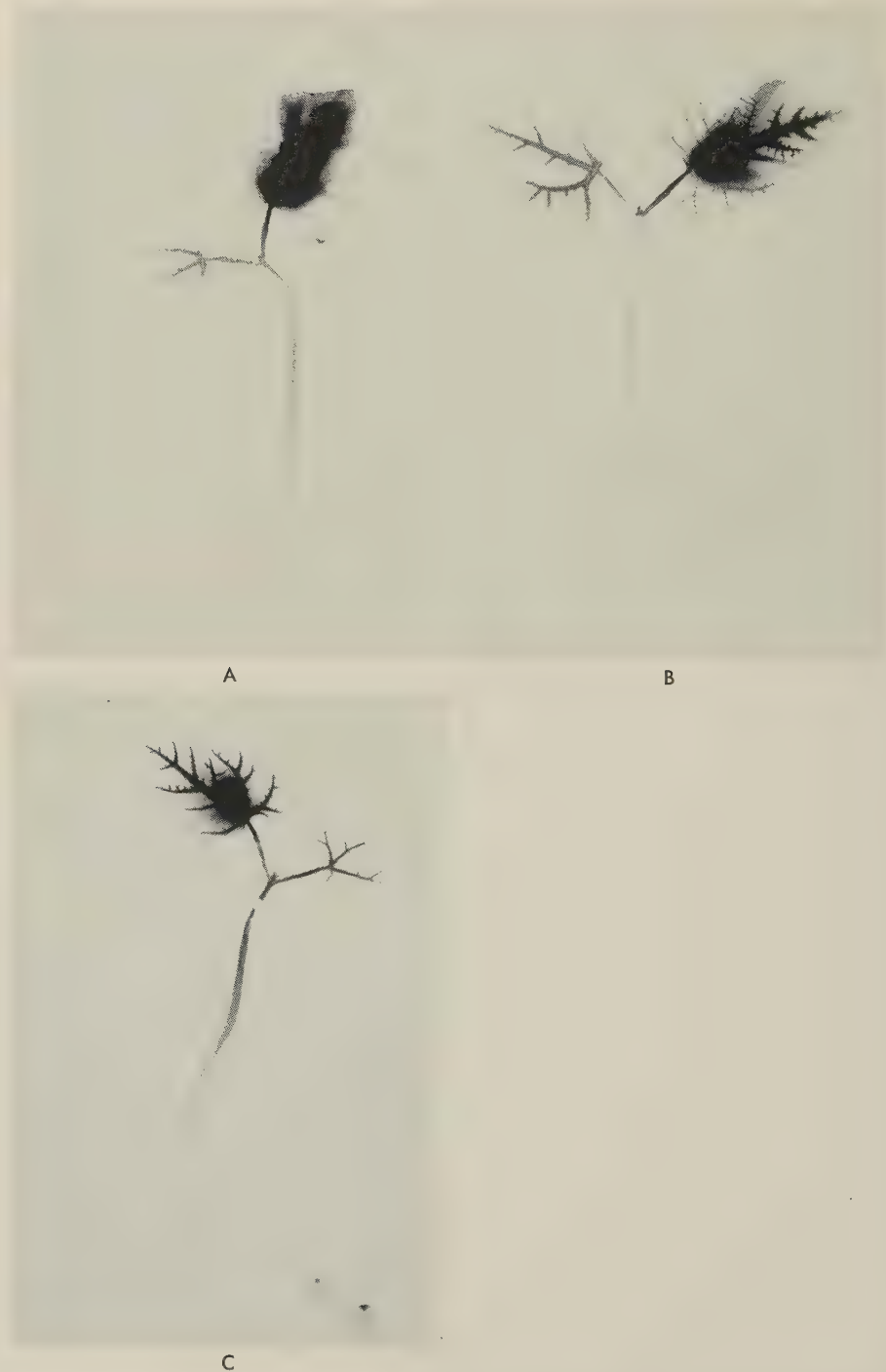


Fig. 19. *A*, radioautograph of bean plant from a humid-chamber treatment. *B*, plant treated on greenhouse bench. *C*, plant from out-of-doors, dry treatment. All plants: dosage 50 μ g, treatment period 4 hours, exposure 2 weeks.



Fig. 20. *A*, radioautograph of a bean plant treated on both leaves. Dosage 50 μ g per leaf, treatment period 4 hours, exposure 2 weeks. *B*, radioautograph of a bean plant treated on one leaf. Dosage 50 μ g, treatment period 4 hours, exposure 4 weeks.

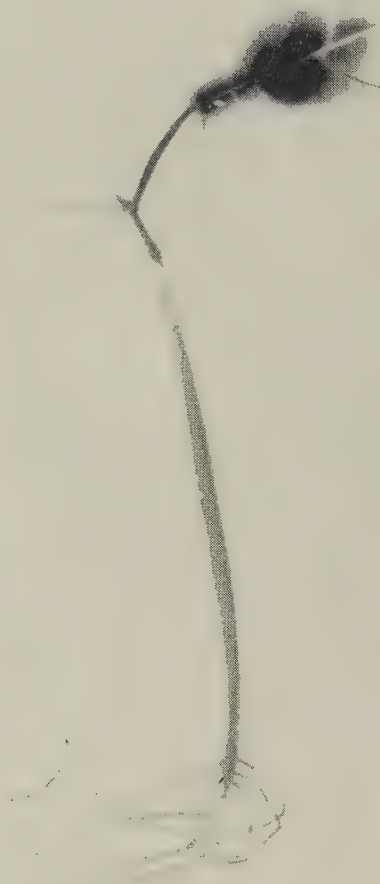
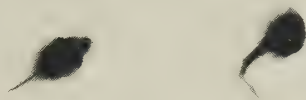


Fig. 21. Radioautograph of a bean plant treated in the center of one leaf over the midrib. Dosage 20 μ g, treatment period 4 hours, exposure 4 weeks.



A

B

Fig. 22. *A*, radioautograph of a bean plant treated on the tip of one leaf. Dosage 50 μ g, treatment period 4 hours, exposure 4 weeks. *B*, similar radioautograph of a plant treated on one lower leaf lobe. Dosage 20 μ g, treatment period 4 hours, exposure 4 weeks.



Fig. 23. Radioautograph of a bean plant that received five 20- μ g treatments around the edge of one leaf. Dosage 20 μ g per treatment (100 μ g total), treatment period 4 hours, exposure 4 weeks.



Fig. 24. Radioautograph of a freeze-dried bean plant. Dosage 5 μ g, treatment period 1 hour, exposure 4 weeks.



Fig. 25. *A*, radioautograph of a freeze-dried bean plant. Dosage 5 μ g, treatment period 4 hours, exposure 4 weeks. *B*, radioautograph of a dry-ice-killed, blotter-dried bean plant. Same dosage, treatment period, and exposure.

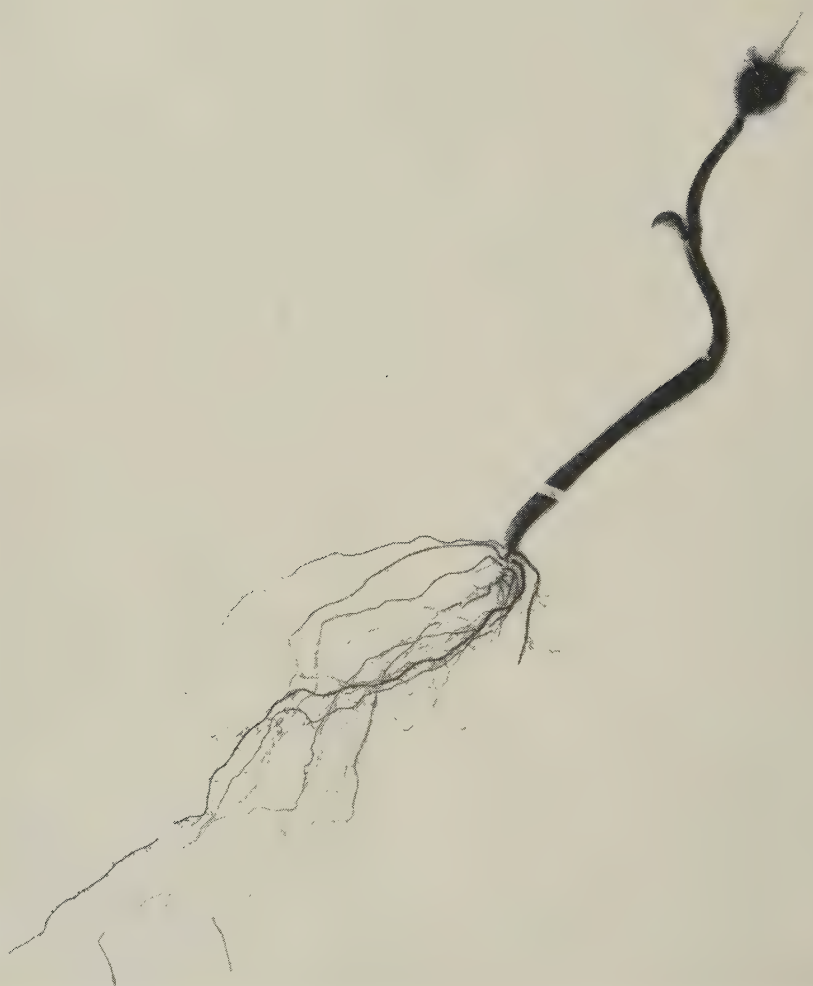


Fig. 26. Radioautograph of a freeze-dried bean plant. Dosage 50 μ g, treatment period 24 hours, exposure 4 weeks.

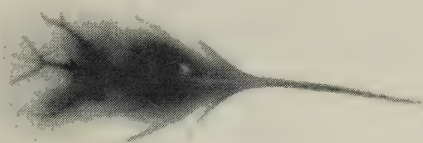


Fig. 27. Radioautograph of a young cotton plant. Dosage 10 μ g, treatment period 4 hours, exposure 2 weeks.

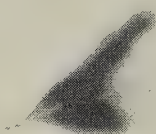


Fig. 28. Radioautograph of a somewhat older cotton plant with true leaves expanding.
Dosage 10 μ g, treatment period 2½ days, exposure 2 weeks.



Fig. 29. Radioautograph of a cotton plant with four expanded true leaves. Treatment on one cotyledon. Dosage 50 μ g, treatment period 4 hours, exposure 2 weeks.

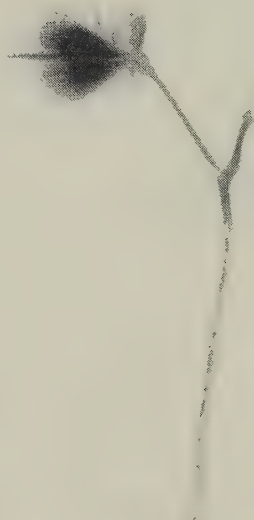


Fig. 30. Treatment on an expanded true leaf. Dosage 10 μ g, treatment period 4 hours, exposure 2 weeks.



Fig. 31. Treatment on the fourth expanding leaf. Dosage 50 μ g, treatment period 4 hours, exposure 2 weeks.



Fig. 32. Radioautograph of a young cucumber plant treated on one cotyledon. Dosage 5 μ g, treatment period 1 hour, exposure 4 weeks.



Fig. 33. Similar plant treated on the first expanded leaf.

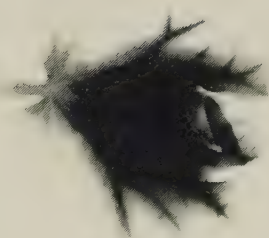


Fig. 34. Similar plant treated on the second expanding leaf.

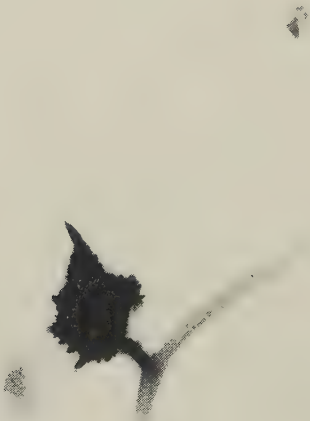


Fig. 35. Similar plant treated on the third expanding leaf. Here the treating solution seems to have flowed down onto the stem.



Fig. 36. Radioautograph of a bean plant killed by quick-freezing and dried in filter paper between warm, dry blotters. Dosage 50 μ g, treatment period 1 hour, exposure 4 weeks.



Fig. 37. Radioautograph of the filter paper upon which the frozen plant of figure 36 was placed for drying.

II. ABSORPTION AND TRANSLOCATION OF 2,4-D BY WILD MORNING-GLORY¹

ALDEN S. CRAFTS²

INTRODUCTION

THE FIRST REPORT of the use of 2,4-D and 2,4,5-T as weed killers in the United States described trials on wild morning-glory (Hamner and Tukey, 1944).³ The formulations contained the parent acids in Carbowax diluted with water, a type of mixture commonly used in the administration of plant regulators. The reported results indicated successful killing of the roots by spraying the tops. Obviously, translocation of the chemicals was involved.

Subsequent rapid development of the use of 2,4-D as a weed killer followed the introduction and sale of the sodium and ammonium salts and the alkyl esters of 2,4-D. The phenomenal growth of this practice is a familiar story. Despite the tremendous popularity of these 2,4-D products, on careful examination the results failed to show any consistent evidence for effective translocation of the materials into the roots of perennial plants. In fact, many investigators were led to question the effectiveness of 2,4-D as an herbicide against perennial weeds.

Something was evidently wrong because the early Carbowax preparation had undoubtedly been effective as a translocated herbicide. Occasional observations of killing of the underwater roots of tules and cattails after treatment with 2,4-D, and a few isolated examples of the undoubted killing of perennial weeds following spraying proved that, under certain conditions, the plant regulators were being transported to roots in lethal doses.

Is there any ready explanation for these facts? From the chemistry of the compounds it is known that 2,4-D acid is low in water solubility, though moderately dissociated when in solution. Compared with the salts, the acid is relatively low in polarity, and higher in lipid solubility than are the ions of the salts. It had been postulated that the penetration of the dinitro compounds into plant cells depends on the concentration of undissociated dinitrophenol molecules in the spray solution (Crafts and Reiber, 1945).

Characteristics of 2,4-D

In the use of translocated herbicides, the concern is not alone with penetration of cuticle and entry into the living mesophyll; migration to and transport within the phloem are additional prerequisites to success. And for this to take place, the toxicant must be water soluble. In 1948, the writer made the statement, "for penetration of the cuticle and absorption by foliage, non-polar compounds should be used" (Crafts, 1948). Considering 2,4-D, the series, ammonium salt, sodium salt, alkylamine salt, alkanolamine salt, is in the direction of increasing polarity, water solubility, and hence conveni-

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³ See "Literature Cited" for citations referred to in text by author and date.

ence of formulation. And for contact action against mustards in a cereal crop, the margin of safety with respect to toxicity to the weeds is so great that little harm results from the decreasing tendency to penetrate.

Alkyl Esters. Considering now the alkyl esters of 2,4-D, the series, methyl ester, ethyl ester, propyl ester, butyl ester, amyl ester, is in the direction of decreasing polarity and hence increasing lipid solubility. This should, and actually does, increase penetration. But for two reasons it works against translocation: (1) the high lipid solubility makes the ester compatible with the cuticle, and hence lowers the tendency for partition from it; and (2) the low water solubility lowers the ester's compatibility with the vacuolar sap and the aqueous phase of the protoplasm of mesophyll cells. The alkyl esters are noted for their high contact toxicity as indicated by dosage recommendations that are usually about half those for salts. But the same properties that induce rapid penetration and violent contact action weaken partition to the mesophyll and vascular tissues, and increase toxicity which quickly knocks out the mechanism responsible for translocation.

Heavy Esters. The shift to the heavy esters came about largely because of injury problems resulting from the volatility of the light alkyl esters. But the introduction of one or more oxygen atoms into the alcohol chain of the ester added to the water solubility of these compounds which, because of their balanced solubility, gave better performance with respect to partition and translocation (Crafts, 1951*a*). Several of the heavy ester formulations have proved superior to the alkyl esters and salts as translocated herbicides on perennial weeds. However, while there are almost unnumbered possibilities for combining alkylene groupings and ether linkages into heavy esters of 2,4-D, certain considerations indicate definite limitations: First, proper balance of the water and lipid solubility must be maintained to insure both penetration and partition. Second, the length or weight of the alcohol chain is limited, for it may come to overshadow the active 2,4-D group and hence lower activity on a straight molecular weight basis (see table 12, page 150, Robbins, Crafts, and Raynor, 1952). And, because the evidence from tests indicates that the esters are not hydrolyzed during penetration, the mass of the molecule may make it unwieldy for ready translocation through the phloem. Finally, the diesters of glycols seem less effective than do monoesters in translocation trials (Leonard, 1954), indicating that this type of molecule will not provide a way out of the dilemma.

The Acid. The acid stands at the midpoint between the salts and the alkyl esters with respect to polarity. The molecule is small and mobile, yet it is not highly volatile. Its chief drawback seems to be its low water solubility from the standpoint of formulation and application. However, this is a virtue in disguise, as seen from the discussion above, for it acts as a buffer to regulate both penetration and contact toxicity. Emulsifiable formulations of 2,4-D acid are available, combining ease of application with a balanced solubility that permits orderly penetration, continued migration to the phloem, and optimum pickup and transport in that tissue. Such formulations should be free of compounds having contact toxicity. They should provide stable spray mixtures that dry down to syrupy films from which absorption can continue indefinitely. And they should provide for adequate spreading and wetting. From results being obtained in current tests of emulsifiable acid formulations, it seems possible that all these prop-

erties may be combined in a single formulation, and that such formulations will give superior results in the field. This explains the success of Hamner and Tukey with the Carbowax formulation of 2,4-D acid.

Application of 2,4-D to Wild Morning-glory

Like so many problems in agriculture, success of chemical weed control depends in part on the material used but also in part on the state or condition or physiology of the plants being treated. This is particularly true in the use of translocated herbicides. Because wild morning-glory, a widespread and important weed, is fairly representative of many such weeds, it was chosen for the studies designed to carry the results of tracer work to practical application in the field. For reasons obvious from the above discussion, 2,4-D acid was used as the test material. In most of the tests, the carboxyl-labeled 2,4-D acid in 50 per cent alcohol solution with 0.1 per cent Nonic 218 was used. Single-drop applications were used in most cases; some involved multiple-drop application; one experiment employed spray application of a solution to plants in the field.

EXPERIMENTAL RESULTS

Experiments with 2,4-D

The work on wild morning-glory had as its objectives a more effective use of 2,4-D as a translocated herbicide and an explanation of some of the obvious failures in the field use of this chemical. It had been shown that there is an optimum value for concentration of 2,4-D applied (H. H. Smith, 1946). Above and below this value, the effectiveness of the herbicide drops off.

An experiment was designed (K. M. Smith, 1950) involving five formulations, four applications, and a series of observation dates. The triethanolamine salt of 2,4-D was to be used in one application of 1,200 ppm, two applications of 600 ppm, four applications of 300 ppm, and eight applications of 150 ppm.

The isopropyl ester was to be applied in similar series. A micronized lot of 2,4-D acid was formulated by suspending the acid in a lubricating oil of S.A.E. 20 viscosity and making a 6 per cent emulsion in water with an emulsifier to provide stability. An acid-in-alcohol mixture was made by dissolving 2.4 gm of 2,4-D acid in 30 ml of 95 per cent alcohol and diluting to 2,000 ml with distilled water. Application was started on July 16, 1949, and subsequent applications were made at weekly intervals.

The amine salt was carried through the whole schedule; the ester was applied once at 1,200 ppm, twice at 600 ppm, and four times at 300 ppm. (At 150 ppm, the plants were dead after five treatments, so that all of the chemical could not be used.) The two acid formulations killed the plants so rapidly that only the 1,200-ppm and 600-ppm applications were made; after three applications at 300 ppm the plants were dead. The ppm in all cases were calculated on the acid equivalent basis so that all plants carried through the schedule received the equivalent of 1,200 ppm of 2,4-D acid. The volume rate of spraying was 100 gallons per acre.

Figure 1 shows the depth of kill produced by the five formulations two months after treatments were completed. These are averages for only six

plants per treatment, hence small differences are of doubtful significance. Since all plants received the same amount of 2,4-D, the larger differences probably relate to the formulations and application methods. Examination of results (fig. 1) indicates that either the amine salt was very slow in entering or it had not translocated at the high concentrations. By the end of the four-week application period the 300-ppm treatment had brought about considerable movement within the plants. The ester, on the other hand, entered rapidly, and the optimum occurred at 600 ppm. Possibly the 1,200-ppm treatment was too toxic, and caused injury in the phloem. The acid in oil gave consistently good results. Acid in alcohol was less effective, and Smith (1950) considered that it had so low a surface tension that it ran from the plant at the 100-gallon-per-acre rate of application. It also dried rapidly, and may have failed to continue to move in from the residue formed. The slopes of the two acid curves indicate that even higher dosages could be used before toxicity would reach a point sufficient to reduce effectiveness. This indicates a regulated absorption and migration in contrast to a rapid accumulation in mesophyll cells resulting from the amine and ester forms.

The fact that the optimums for the salt and ester curves are at different points indicates the possibility that different forms of 2,4-D may have different concentration:volume relations. We know from experience that they have different dosage relations—for instance, the esters versus the salts. This difference in the optimum concentration is probably directly related to the relative rates of absorption and migration within the leaf, as these determine accumulation of 2,4-D within living cells.

Smith's (1950) acid-in-oil treatment involved the deposit, on the leaves, of a suspended, micronized 2,4-D acid in an oil film. Absorption in this case should be slow and regular, depending upon the solubility of the acid in the cuticle and its migration into living cells. The American Chemical Paint Company distributed a number of samples of micronized 2,4-D acid under the code designation of LFN472. After much field testing, the company has abandoned this for the emulsifiable acid formulation, Weedone 638. However, the Fruitgrowers Chemical Company, Limited, of New Zealand is still testing suspended acid formulations of both 2,4-D and 2,4,5-T, and the Stauffer Chemical Company is producing 2,4-D and 2,4,5-T acid pastes for use in the suspended form. Excellent results have been obtained with these products under certain conditions, and it seems possible that they may yet find a place among our weed-control chemicals.

Smith (1950) performed one additional experiment that is of interest. Using bean plants, he applied a 40- μ g dose of acid to a single unifoliolate leaf in one droplet, two droplets, and four droplets. Figure 2 shows the relation between curvature of the bean plants and area over which the given dose was dispersed. Apparently the 40- μ g dose at 1,000 ppm 2,4-D acid was not toxic, and the average bending of six replicates was over 60°. When the dose was placed over a larger area in two drops, the angle was about 30°. With four drops, on an even larger area, the angle was less than 20°. This bears out the conclusions of Loomis (1949), Smith (1946), Fisher (1952), and Crafts (1953, 1956) with respect to the efficiency of coarse versus fine droplets in spray application.

With the above experimental work and wide field experience as a back-

ground, a project was initiated involving the use of labeled 2,4-D as a translocation indicator. In addition to the studies already described, on beans as test plants (Crafts, 1953, 1956), tests were carried out on wild morning-glory plants in the greenhouse and in the field.

Experiments with 2,4-D*

Seedling Studies. The first experiment involved a time series on morning-glory seedlings in the cotyledon stage. Greenhouse-grown plants were treated by applying 10 μ g of 2,4-D* in 50 per cent ethyl alcohol on one cotyledon in the form of a droplet of 0.01 ml. The treatment times were $\frac{1}{4}$, $\frac{1}{2}$, 1, 2, and 4 hours. The plants were killed between blocks of dry ice, dried between warm, dry blotters, and autographed for one week. There were five plants per treatment.

While there was undoubtedly some movement of 2,4-D in the xylem of these seedlings during drying (Crafts, 1956), the consistent differences with time indicate that this was not serious. Average movement in the $\frac{1}{4}$ -hour treatments was less than $\frac{1}{2}$ inch; for the $\frac{1}{2}$ -hour treatments, about $\frac{3}{4}$ inch; for the 1-hour treatment, $\frac{3}{4}$ inch; for the 2-hour treatment, $1\frac{3}{4}$ inches; and for the 4-hour treatment, $1\frac{1}{4}$ inches. The roots of these seedlings that could be washed free of soil and carried through the drying process averaged $2\frac{1}{4}$ inches in length. Figure 3 shows the autograph of three plants treated for 2 hours. Evidently absorption and translocation were taking place in these seedlings, but the velocity of movement was not high. Included in this experiment were some trials on larger plants, and in these translocation was more extensive. Five plants in the 2-leaf stage averaged downward movement of 2 inches in 4 hours; four of the plants had upward movement from the cotyledon into the growing shoot. Five plants having four true leaves each averaged 4 inches downward movement in 4 hours. Some larger plants having a foot or more of growth showed variable amounts of movement. One showed strong movement both upward and downward from a cotyledon that was still green and healthy; a similar plant showed no movement from a young upper leaf. The treating solution used in this and the following experiment did not contain a surfactant, and this may be one reason for the wide variability among plants.

The second experiment involved a 4-hour treatment time, with 1 μ g of 2,4-D* in 50 per cent ethyl alcohol, on plants in the cotyledon, 2-leaf, 5-leaf, and 10-leaf growth stages. Eight plants in the 10-leaf stage, treated on a lower leaf, showed no movement beyond the treated leaf. Results on 11 plants in the 5-leaf stage were variable, five plants showing some transport and six plants none. Eighteen out of 20 plants treated in the cotyledon stage showed some transport. The low dosage, the lack of a surfactant, the short exposure on the film (1 week), and the high light intensity and low humidity prevailing may all have contributed to the general lack of absorption and translocation in these experiments.

The third experiment involved use of a test solution of 2,4-D* in 50 per cent ethyl alcohol containing 1 per cent Nonic 218. The dosage was 1 μ g; the treatment time was 8 hours. Twenty plants were treated: five in the 6-leaf stage treated on an upper leaf; five in the same stage treated on a

* The symbol 2,4-D* indicates the radioactive form of 2,4-D. The 2,4-D* used had C¹⁴ in the carboxyl position. It was purchased from Tracerlab.

cotyledon; five in the 12-leaf stage treated on a tip leaf; and five similar plants treated on a cotyledon. The 6-leaf plants treated on a tip leaf averaged 1 inch of movement down the stem. The leaves were from half to full grown. Similar plants treated on the cotyledon had 2,4-D* well distributed throughout the upper root systems. All plants transported 2,4-D*, the average distance being $5\frac{1}{2}$ inches, the greatest, 8 inches, with movement into lateral roots in several instances. In the 12-leaf plants, treatment of tip leaves resulted in even more restricted movement than in the 6-leaf plants. Figure 4 is representative of plants treated on a tip leaf. Cotyledon treatment resulted in extensive distribution in the roots in four of the five plants (fig. 5).

The next experiment involved 30 plants in the 6-leaf stage and 12 plants in the 12-leaf stage. Treatment was for 4 hours with 1 μ g of 2,4-D* in 50 per cent alcohol solution containing 1 per cent Nonic 218. The dried plants were exposed on the X-ray film for 2 weeks. Ten 6-leaf plants treated on a small tip leaf showed movement beyond the petiole in only one case and for a distance of less than 1 inch. Treatment of ten 6-leaf plants on a leaf situated near the middle of the plant resulted in extensive movement in the stems of eight out of 10 plants, and in four of the eight there was some 2,4-D* in the roots. Figure 6 shows one such plant. Treatment of ten 6-leaf plants on a basal leaf resulted in movement into the roots of seven plants. In four of these there was some upward movement in the stem. In the 12-leaf set, there was no transport out of tip leaves. Four out of five plants in this set treated on middle leaves showed considerable movement; and four out of five treated on a lower leaf had 2,4-D* in the basal stems and roots. Transport was not extensive in any of these plants, but the dosage was only 1 μ g. From the nature of the autographs it seems apparent that some of the tracer is retained in living cells along the route of transport. This brings about a continuous diminution in concentration which, with such small applications, probably limits the extent of movement.

One further experiment was conducted (July 9, 1952) with 2-leaf and 4-leaf seedlings, exploring the possibilities for movement from upper leaves and cotyledons. Vigorous seedlings growing in the greenhouse were used, and some excellent autographs were obtained. The treating solution contained 1 per cent Nonic 218, the dosage was 1 μ g, and the test period, 4 hours. The plants again were killed between blocks of dry ice, and dried between warm, dry blotters. The exposure on the X-ray film was two weeks. Of 10 plants in the 2-leaf stage, four treated on young, expanding tip leaves had 2,4-D* in the treated blade and petiole. Of six treated on larger leaves, two had the tracer well down the stems, four had it into the roots. Figure 7 illustrates some of the latter. Of 10 similar plants treated on the cotyledons, all had the tracer in the roots, and in three it was also present in tip leaves. These seedlings illustrated very well the possibility for two-way movement from a leaf that was actively transporting (fig. 8a and b). Considering now the 4-leaf plants, of 10 treated on tip leaves, five contained 2,4-D* in the roots (fig. 9a), three had it in the stems only, and two in petioles and treated leaves (fig. 9b). Of 10 plants in the 4-leaf stage, treated on their cotyledons, eight had the tracer well into the roots, two had it only in the treated leaves and petioles.

Because we suspected that some of the variability in response found in

these experiments might have resulted from contact injury by the test solution, a series of applications was made on morning-glory leaves at various positions on the plant. These consisted of alcohol alone and with 1 per cent and 0.1 per cent Nonic 218. It was found that the 50 per cent alcohol being used caused no injury when applied in 0.01-ml droplets. When 1 per cent Nonic 218 was included there was some injury to young leaves at the tips of the plants; 0.1 per cent Nonic 218 caused only slight damage to very young leaves, none to expanded leaves. As a result of this test, the 2,4-D* solution being used was changed to contain 0.1 per cent Nonic 218; the 50 per cent alcohol concentration was retained.

The next experiment was designed to find if there were any significant differences in the distribution of 2,4-D* attributable to the method of killing. Large, greenhouse-grown plants were used. Five were cut into six portions at the end of the 4-hour treatment period and then quick-frozen, thawed, and dried between warm, dry blotters; five were cut while frozen; five were cut after thawing. Ten were killed and dried without being cut into fractions, and of these, five were killed after a 4-hour treatment time and five were killed immediately after treating. Of the 15 plants cut before, during, and after freezing, none showed extraordinary distribution of the 2,4-D*. Evidently, when the treatment time was 4 hours, the artifact of xylem movement upon thawing was not causing abnormal movement. There was no extensive transport into tips or roots in these plants.

In the five plants having zero treatment time, the most extensive movement was for a distance of about 4 inches in each direction from the treated leaf of one plant. Two other plants had less extensive movement, two had none at all. Of the five contrasting plants with 4-hour treatment time, one showed transport for about 22 inches into the roots, two moved the 2,4-D* 20 inches, one transported it 4.5 inches, and one carried it only 1 inch into the petiole.

An experiment using the same solution and dosage, a 4-hour exposure period, and killing with dry ice, tested movement in plants in the bud stage, in the blossoming stage, and in a stage having full-grown seeds. In the plants treated in the bud stage, two out of three had 2,4-D* in the young growing tip, and the third had some upward movement (fig. 10). All three had some transport in the basipetal direction, but only one had 2,4-D* in the roots. Of 10 plants treated in the blossoming and seed stages, none had 2,4-D* in the tips, but seven had some upward movement. Downward movement predominated in these more mature plants, all having the tracer to a distance of 4 inches or more, five having it over 6 inches, and two having it present in the roots.

The final greenhouse experiment with morning-glory attempted a comparison of four surfactants. The results were inconclusive because of lack of uniformity in the plants. Movement was upward in some, downward in others, and completely lacking in several. These were large plants in pots. Probably small seedlings would be better for such testing, with at least 10 replicates used for each material.

Field Experiments. A number of experiments using 2,4-D* as a translocation indicator were conducted in the field.⁵ The first experiment was performed on May 19, 1952, and the second on May 30. A third was started

⁵ Mrs. Barbara Kean conducted these experiments.

on September 24, and three were carried out in October of the same year. Thus it is apparent that these experiments covered two rather distinct climatic situations and that they represented different stages of development of the plants. These facts, considered in connection with the age of shoots and the location of the treated leaves, give clues for interpreting the results obtained. In all cases, 0.01-ml droplets of the test solution, containing 5 μ g of 2,4-D*, applied to individual leaves constituted the testing method. No wetting agent was used in the first two experiments. The plants were dug from the ground, killed with dry ice, and dried between warm, dry blotters. Autograph exposure was for one week.

The first experiment involved some young shoots coming up from old roots in a field that had been disked to destroy annual weed growth. The leafy shoots aboveground were from 4 to 12 inches long; below the soil level they came off the roots at a depth of around 8 inches. No buds or blossoms were present. All shoots were treated on a lower leaf; the treatment periods were 2 and 4 hours. Of 25 shoots treated, only one showed upward movement and that for only 1½ inches. Twelve shoots had some downward movement, their average being 5.5 inches. Evidently these young vegetative shoots were still growing at the expense of the underground storage roots and had scarcely started replenishing the root reserves. The 4-hour period resulted in greater movement, with 10 plants out of 15 averaging 5.9 inches. The 2-hour period resulted in transport in three out of 10 plants, with an average distance of 4 inches.

In the second field experiment, 20 plants from the same location were treated on May 30. Ten of those were in the blossoming condition, and all such were treated on a lower leaf. Ten were treated in the preblossoming stage, five on an upper leaf and five on a lower leaf (fig. 11). The treatment period was 8 hours. Here the physiological condition of the plants had a profound effect on the results, and the effects bear a definite relation to field observation of commercial spraying. Of the 10 blossoming plants, only one showed any movement, and that for only 1½ inches in a basipetal direction. Of the preblossoming plants, all displayed some movement—those treated on an upper leaf averaged 4 inches downward and 1.2 inches upward; those treated on a lower leaf averaged 13.2 inches downward and 1.6 inches upward. These results agree with the common recommendation to spray wild morning-glory with 2,4-D in the prebloom stage. Later treatment often fails to kill the roots to any appreciable depth. Apparently, during blossoming, food materials are largely used locally in the production of flowers and seeds. Only later, when the seeds are mature, does downward transport resume, and at that time if the roots are mature they fail to react to 2,4-D.

By the time field testing was resumed in the fall, a test solution had been standardized—500 ppm of 2,4-D* in 50 per cent alcohol, so that 0.01 ml gave 5 μ g. Nonic 218 was used at a concentration of 0.1 per cent. Dry-ice killing was continued as it apparently gave no artifact following the 4-hour treatment time. Exposure on the X-ray film was 4 weeks.

The first experiment in the fall used 14 plants, eight growing in a dry field and six on an irrigation ditch where there was ample moisture. All shoots were treated in a prebloom stage on a middle leaf. All plants in the dry condition moved the 2,4-D* downward for an average distance of 7.4 inches (fig. 12a, b); two moved it upward, one for 6 inches (fig. 13) and

one for 1 inch. All plants in the moist situation moved the 2,4-D* downward for an average of 10 inches; five out of six moved it upward for an average of 1.6 inches (fig. 14). From these results it seems that available moisture promotes the translocation of 2,4-D*. However, plants in a dry situation move the chemical downward, and lack of response in such cases probably reflects lack of response at the site of ultimate action rather than lack of transport into the roots. This same conclusion is borne out by further work.

An experiment performed on October 1, 1952, utilized 15 plants, five in the bud stage, five in bloom, and five with seeds forming. Treatments were near the tops of shoots. All plants but one in the blossoming stage transported the tracer in a basipetal direction; seven moved it upward. Movement was most rapid and prominent in the stems of the plants in the seeding stage.

A similar experiment on October 8, treating in the middle of shoots, gave an average transport downward of 7.4 inches for the plants in bud, 5 inches for the plants in bloom, and 10.4 inches for those forming seeds. One plant in bloom moved the tracer 1 inch upward; one in seed moved it 4 inches upward.

A third test on October 9, treating at the base of shoots in bud, in bloom, and in seed, gave the following figures: shoots in bud, downward 8.6 inches, upward 0; shoots in blossom, downward 5.4 inches, upward 0.8 inch; shoots in seed, downward 3.6 inches, upward 0.2 inch.

It is apparent that these morning-glory shoots were actively transporting foods and that 2,4-D* moved where the foods were going. Movement was predominantly downward; it was more active in plants in the bud and seed stages than in those in blossom; it took place in plants in dry as well as moist situations.

A study of 2,4-D* translocation in morning-glory growing in the field was begun in August, 1953.^o A site on Yolo sandy loam was chosen, to facilitate digging of the roots. One patch of morning-glory was irrigated with a sprinkler until the soil was moist to a depth of 2 feet. A similar unirrigated plot was used where testing proved that there was available water at a depth of 5 feet. A trench 4 feet deep was dug, and a trowel and ice pick were used to remove the individual roots. In this way, roots were excavated to depths of 5 feet or more.

On August 3 and 4, plants in the two situations (moist and dry) were treated with 5 μ g of 2,4-D* per application. The solution was in 50 per cent alcohol, and included 0.1 per cent Nonic 218. This test solution was made up from a batch of 2,4-D* containing 1.27 millicuries of activity per millimol. A preliminary run was made on August 3, a comprehensive experiment was started August 4, and collections continued until August 6. The plants were killed with dry ice, dried between blotters, and left on the X-ray film for 4 weeks. The preliminary test gave variable results—one plant transported 2,4-D* to a depth of 51 inches in the root system during a 75-hour period of treatment. This was the total depth to which the root was removed; the tracer probably extended somewhat farther down.

The test started on August 4 involved 18 plants, nine in the wet and nine in the dry situation. One in each situation was treated on a tip leaf and given a treatment period of 4 hours, another was treated for 27 hours,

^o Under the direction of J. E. Pallas, Jr.

and a third for 48 hours. Similar groups of three were treated on a leaf in the center of the plant and on a basal leaf.

The most significant difference related to the period of treatment. The average distance of transport in the 4-hour period was 11 inches, for the 27-hour period, 30 inches, and for the 48-hour period, 39 inches. A number of roots in the longer period treatments had 2,4-D* to their lower extremities; 68 inches was the greatest length of root excavated, and it autographed to the end.

The average distance of transport for all time groups in all the treatments in wet soil was 32.5 inches; in dry soil, 23.9 inches. However, variation among the plants in wet soil was from 0 to 68 inches, among those in dry soil, from 0 to 54 inches. Differences between treatments on tip, middle, and basal leaves were not significant. Considering that these were all fairly mature plants with available soil moisture, even in the dry situation, it seems logical that they should have behaved alike. Transport evidently continued for more than 4 hours, and in some instances extended to the full depth of excavation. One observation that was evident from the previous experiments was repeated here several times: 2,4-D* transport is not limited to vertical roots. In several instances, as noted in figures 15 and 16, the tracer moved down a rhizome until it merged with a horizontal rhizome or root. In nearly every case of this type, movement continued along the horizontal structure. This contradicts interpretation of field tests to the effect that 2,4-D moves by polar transport and does not enter horizontal laterals.

One additional experiment on morning-glory was conducted in the field during the 1953 season. In this, 0.5 ml of the 500-ppm stock solution of 2,4-D* (1.27 mc per mM) was mixed with 0.145 ml of Weedone 638⁷ and made up to 77.7 ml. This was applied to 1 square yard of a small patch of morning-glory foliage in the field. The application was designed to deliver $\frac{3}{4}$ pound of 2,4-D per acre with 5 μ g of 2,4-D* per average plant. Figure 17 shows one of the treated plants. Square-yard areas in the wet and dry situations mentioned above were treated, and plants were collected, killed, and dried after periods of 4 hours, 24 hours, 27 hours, 48 hours, 72 hours, 8 days, 2 weeks, 3 weeks. Exposure on the X-ray film was 4 weeks.

Careful evaluation of the results of this study shows that the only factor that produced significant differences was treatment time. The 4-hour treatment averaged 16.5 inches of transport; the 24- and 27-hour treatments combined gave a value of 32.4 inches; the 48-hour period gave 39 inches; and the 72-hour period, 45.2 inches. The 8-day, 2-week, and 3-week treatments gave transport values that totaled the entire lengths of the excavated roots and were therefore not averaged (fig. 18). The dry treatments gave the more rapid transport in the 4-hour period, but with longer treatment the plants from the irrigated plot had the more extensive movement. However, the plants per treatment were too few to give significant differences. The important point is that roots were impregnated with 2,4-D* to the depth of 5 feet or more by 72-hour treatments and all longer periods of treatments. Again, several plants used in these tests produced evidence for movement into horizontal lateral roots (fig. 19).

⁷ An emulsifiable acid formulation of 2,4-D manufactured by the American Chemical Paint Company, Ambler, Pennsylvania.

DISCUSSION AND CONCLUSIONS

While these studies on the use of radioactive 2,4-D have not produced new and startling information, they give a fairly comprehensive view of 2,4-D transport in plants and they have confirmed a number of field observations on 2,4-D response of wild morning-glory, thus strengthening the convictions behind our current recommendations.

Interpretation of much experimentation suggests that 2,4-D moves with foods in plants (Crafts, 1951b). If this is true, then certain relationships must be apparent: (1) 2,4-D transport must be preceded or accompanied by photosynthesis or hydrolysis and movement of reserve foods. (2) 2,4-D should move out of mature leaves, but should remain in young leaves that are importing foods. (3) 2,4-D should move from lower leaves to roots, from upper leaves to growing shoots or fruits. And as a corollary it may move in both directions from median leaves. (4) Depending on the growth of the plant, movement of 2,4-D at times should be predominantly downward into roots, at other times it should be predominantly into growing shoots, and sometimes it should be predominantly into flowers, fruits, and seeds. (5) If 2,4-D movement accompanies the movement of foods, there should be no polarized movement in vascular channels in the sense of the polar basipetal movement of IAA in the oat coleoptile. Having set up this series of generalizations, it should be of interest to check the present results to see how well they agree.

Although no specific tests on starved plants have been run on wild morning-glory (see, however, experiments on beans reviewed [Crafts, 1951b] or described [Crafts, 1956]), certain preliminary work with seedlings indicated that favorable illumination is required to bring about translocation of 2,4-D*, and even then some plants may not respond within a given short transport period.

Many autographs prove that 2,4-D* will not move out of young tip leaves of well-developed plants. It moves readily from mature, green cotyledons and from full-grown leaves. Movement to roots in well-developed plants is greater from basal than from tip leaves. Movement to growing tips, flowers, and fruits may take place from any well-developed leaves. Two-way movement was observed in a number of plants. In some, movement to growing tips took place at such low concentration that only the tip would autograph; the intervening stem was too low in activity to expose the film.

Movement from cotyledons is initially into roots; after the shoot has grown a few inches, movement may take place both downward and upward. From cotyledons, it is always predominantly downward. Considering transport in shoots growing from old established roots, movement fails from the early, rapidly expanding leaves; at the bud stage it is predominantly downward into the roots; at the flowering and early fruiting stages it is into the flowers and fruits, and much less strongly downward; in the ripe seed stage after end growth has ceased it is again predominantly downward. Failure of spray treatment at this stage is apparently not the result of failure of transport.

Every test involving movement from a vigorous shoot down the stem and rhizome to a horizontal lateral resulted in lateral movement. Apparently, polar movement of 2,4-D* in a directional sense is nonexistent in morning-

glory. The only directional effect is from regions of food synthesis to regions of rapid food utilization.

If transport is active in mature morning-glory in the ripe seed stage, and also in horizontal laterals, why does late summer spraying fail and why do lateral roots so often survive the spray treatment? The answer seems to be in the final response of the root tissues to 2,4-D. Dr. van Overbeek pointed out (1947) that 2,4-D was most active against meristematic tissues. While morning-glory roots are young, growing, and meristematically active they respond. After they have matured and stopped growing, starch is being stored in mature cortical cells, and the roots do not respond. Hence, although absorption and translocation take place normally at those stages, spray treatment fails.

In considering the development of morning-glory from old established roots, it is apparent why lateral roots survive. In the spring, all roots have plenty of available soil moisture, and root growth is very active. However, the tops are importing foods from the roots, and the main stream of assimilates is flowing in the wrong direction. In the bud stage the roots are still active and the tops are re-storing foods in the roots; treatment is successful and all roots respond. Soon, however, moisture in the topsoil begins to become deficient; lateral roots mature and cease end growth; starch storage utilizes the foods. Lateral roots fail to respond, but vertical roots and deeper laterals are killed. As soil moisture limits root growth to greater depths, killing of roots fails and treatment is ineffective. If water is supplied by irrigation, root growth may start again and treatment will be successful provided the top growth is still green and healthy.

These growth and soil moisture relations seem to explain why morning-glory may be killed throughout the summer in the moist coastal belt of California, whereas in the dry interior valleys it responds best during the bud stage in early summer and again in the autumn only if top growth is vigorous and soil moisture is available. Failure in the case of green, vigorous plants growing in irrigated soil in summer may result from too rapid drying of the spray or too rapid killing of tops by contact action of poor formulations. In such situations, use of the emulsifiable acid is indicated, and tests have proved its superiority.

In some of the seedling treatments using 1 μg of 2,4-D, the concentration of the tracer seems to be fully as high in the stem as on the treated leaf (figs. 6, 7, p. 353). This indicates that there is an active process involved in the absorption; a diffusional process would result in a gradient. Reinhold (1954) has shown that IAA absorption involves an active process, and possibly 2,4-D at low concentrations is taken in by the same mechanism.

If an active process is responsible for 2,4-D uptake by leaves, it seems possible that prolonged absorption of the material at low concentration might result in much greater accumulation than would absorption from a toxic concentration that soon kills the leaf. A good many observations from the field bear out this deduction, and it would seem advisable to give the proposition more thorough testing. Smith's (1950) tests reported in this paper involved too long time intervals (one week). From our work with 2,4-D* it seems possible that 8- or 12-hour intervals would be better. Such slow absorption was the thought behind the suggestion of using suspended 2,4-D acid formulations. These have not proved entirely satisfactory, but

as noted (page 338), they are still undergoing test. Incorporation of a heavy white oil has aided in the absorption of micronized 2,4-D acid so that it approaches the esters in effectiveness. Possibly by altering particle size, and including proper surfactants and filming agents, the slow, regulated absorption of 2,4-D acid from a solid phase deposit may yet prove to be a superior method for handling some of the more refractive perennial species.

From the above considerations it should be apparent that formulation of 2,4-D herbicides for use on perennial weeds is important and that, even with the best formulations, knowledge of the physiology of the plants is required. This is the story that evolves from the use of radioactive 2,4-D as a translocation tracer in such plants.

SUMMARY

In the use of 2,4-D against perennial weeds, formulation is important. Emulsifiable acid and heavy ester formulations are proving superior to the older salts and light esters.

Droplet treatments with 2,4-D* on greenhouse-grown seedling morning-glory plants produced satisfactory radioautographs. Surfactant in the formulation increased absorption and translocation. From cotyledons, movement was downward into roots; from middle leaves, it was downward or both upward and downward; there was little or no movement out of very young, expanding tip leaves. Some tracer was retained in living cells along the route of transport.

Tests on field-grown plants proved that 2,4-D* can be used as a tracer in the field. Young vegetative shoots from old roots treated in May were not highly active; only 12 out of 25 treated shoots moved the tracer downward; the distance averaged 5.5 inches. In a later test, 10 preblossoming plants and 10 blossoming plants were treated. Five preblossoming plants treated on an upper leaf moved the tracer down an average of 4 inches; on a lower leaf, 13.2 inches. Of 10 blossoming plants, only one moved the tracer—downward 1.5 inches.

Tests in September and October proved that 2,4-D* was moved most actively in plants growing in moist soil. Movement was most rapid and extensive in plants in the seedling stage. These tests indicate that 2,4-D* moves where foods are moving in morning-glory plants.

A group of large plants growing in a Yolo sandy loam was used in August, 1953, for tracer tests. After treatment, roots were dug to depths of 5 feet or more. Treatment time was the predominant factor determining depth of transport. After a 4-hour period, movement was 11 inches; after 27 hours, 30 inches; after 48 hours, 39 inches. In some of the latter plants, 2,4-D* was found in the lower extremities—in one, to a depth of 68 inches. Transport was not limited to vertical roots. Plants sprayed with an emulsifiable acid formulation, with 2,4-D* added, carried the tracer to the full depth of excavation in 8-day, 2-week, and 3-week periods.

ACKNOWLEDGMENTS

Several people were responsible for the experiments described in this paper. Mrs. Barbara Kean, Charles J. McCarthy, and James E. Pallas, Jr. planned the work, treated the plants, and prepared the autographs. Some early work

by Kenneth M. Smith (1950) in partial fulfillment of the requirements for the Master of Science degree is cited as background material.

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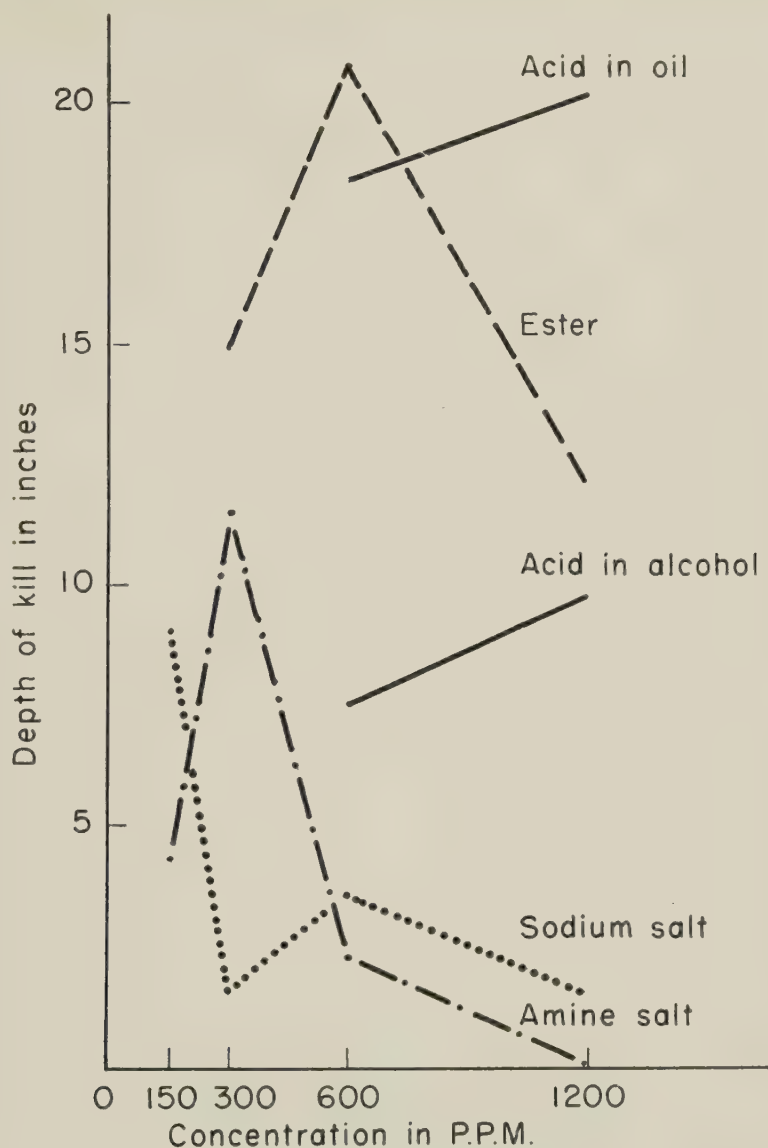


Fig. 1. Depth of kill produced by five different 2,4-D formulations, shown in inches as an average of six plants. Concentrations are shown for the individual applications. With the 150-ppm plots excepted, the total amount applied was, in all cases, 1,200 ppm.

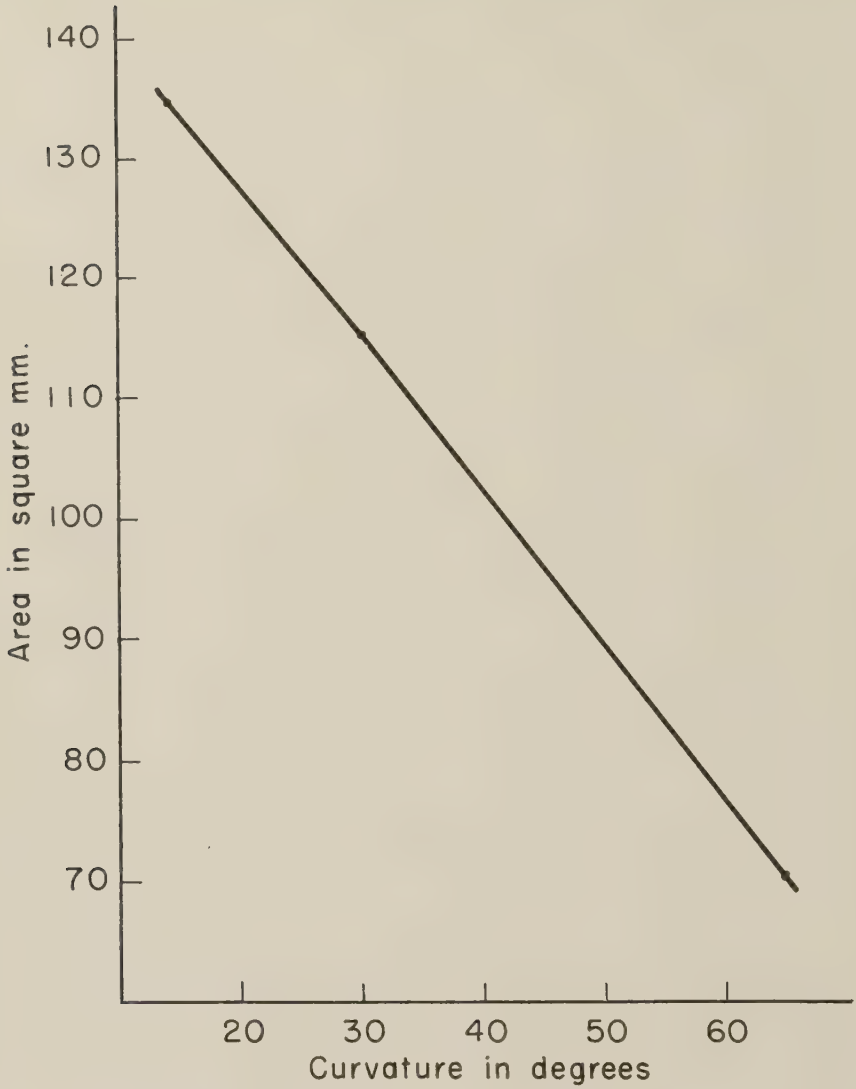


Fig. 2. Effect of area exposed to the herbicide on the intensity of plant response. Total amount of herbicide was, in all cases, the same; concentration was, in all cases, 1,000 ppm. Area covered was altered by applying in one drop, two drops, and four drops. Response is indicated by degree of curvature.



Fig. 3. Radioautograph of wild morning-glory seedlings, each treated on one cotyledon for a period of 2 hours. Dosage, $10\text{ }\mu\text{g}$ of 2,4-D*. Exposure, 1 week.



Fig. 4. Radioautograph of plant treated on a tip leaf that was actively growing. Evidently this leaf was still importing foods. Dosage, $1\text{ }\mu\text{g}$ with 1 per cent Nonic 218. Exposure, 1 week. Treatment period, 8 hours.



Fig. 5. Radioautograph of a plant treated on one cotyledon. Dosage, 1 μ g of 2,4-D* with 1 per cent Nonic 218. Exposure, 1 week. Treatment period, 8 hours.

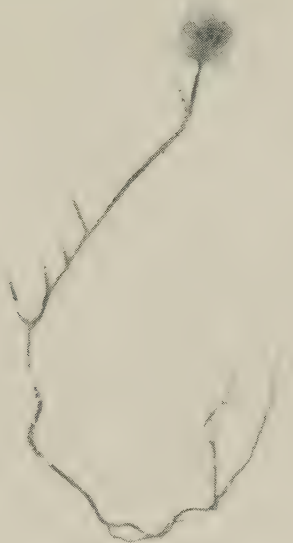


Fig. 6. Radioautograph of a 6-leaf plant, treated on one middle leaf, showing extensive movement of 2,4-D* into the roots. Note uniform distribution of 2,4-D*. Dosage, 1 μ g with 1 per cent Nonic 218. Exposure, 2 weeks. Treatment period, 4 hours.



Fig. 7 *A, B, C, and D.* Radioautographs of young morning-glory plants treated on young leaves. Plants *A* and *B* illustrate lack of transport from very young leaves; plants *C* and *D* show transport from more developed leaves. Note, in the latter, the uniform distribution of 2,4-D* within the treated plants, indicating active transport rather than a purely diffusional movement. Dosage, 1 μ g with 1 per cent Nonic 218. Exposure, 2 weeks. Treatment period, 4 hours.

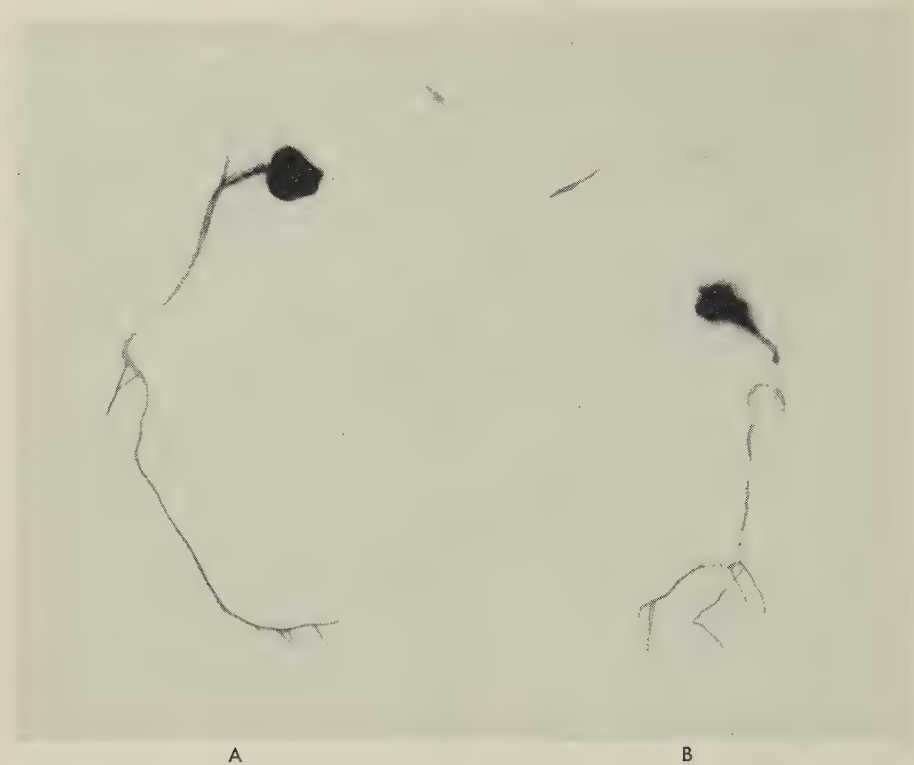


Fig. 8 *A* and *B*. Radioautographs of young morning-glory plants treated on one cotyledon. Movement in these has been two-directional. Dosage, 1 μ g with 1 per cent Nonic 218. Exposure, 2 weeks. Treatment period, 4 hours.

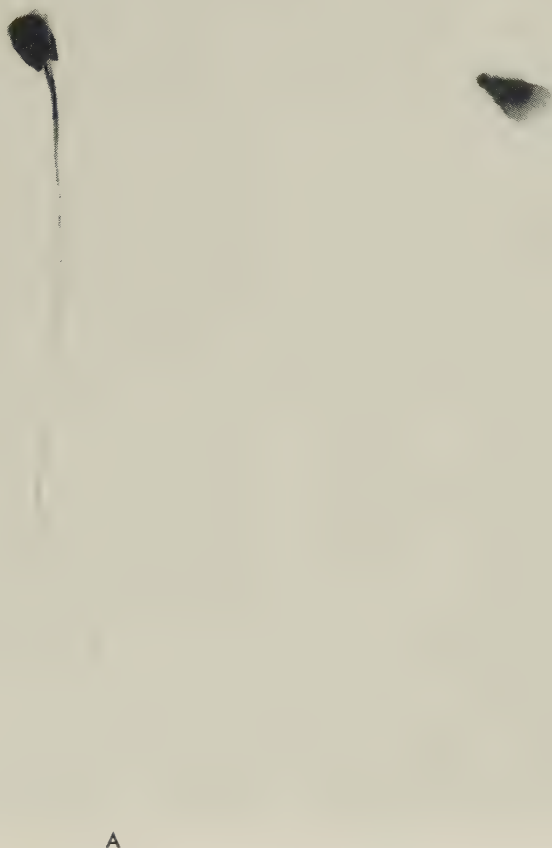
**A****B**

Fig. 9 *A* and *B*. Radioautographs of young morning-glory plants treated on a tip leaf. *A*, movement from a fully expanded leaf into the roots; *B*, retention of 2,4-D* in the treated leaf. Dosage, exposure, and treatment as in figure 6.



Fig. 10. Radioautograph of wild morning-glory plant treated on a middle leaf in the bud stage. Movement of 2,4-D* was predominantly upward, and a slight activity can be seen in the young tip of the stem; the image is too light, however, to reproduce. Dosage, 5 μ g with 0.1 per cent Nonic 218. Exposure, 4 weeks. Treatment period, 4 hours.



Fig. 11. Radioautograph of field-grown morning-glory plant treated on May 30, 1952. Plant was in the preblossoming stage, and treatment was on a lower leaf. Dosage, 5 μ g with no surfactant. Exposure, 4 weeks. Treatment period, 8 hours.



Fig. 12 *A* and *B*. Radioautographs of two field-grown plants from a dry location. Date of treatment, September 24, 1952. Treatments were on middle leaves in the preblossom stage. Dosage, 5 μ g with 0.1 per cent Nonic 218. Exposure, 4 weeks. Treatment period, 4 hours.



Fig. 13. Plant treated as in figure 10. Movement here is both upward and downward.



Fig. 14. Radioautograph of a plant from an irrigation ditchbank. Movement here is both upward and downward.



Fig. 15. Radioautograph of plant treated on a lower leaf, August 4, 1952. Note the two-way movement along the horizontal root. Dosage, 5 μ g with 0.1 per cent Nonic 218. Exposure, 4 weeks. Treatment period, 27 hours.



Fig. 16. Plant treated as in figure 13 except that the treatment period was 48 hours.



EXPT. 3
24D/ACRE
not f.e. Irrig. 4 HRS

Fig. 17. Radioautograph of a sprayed plant. Dosage, approximately 5 μ g.
Exposure, 4 weeks. Treatment period, 4 hours.



EXPT. 3
1/4 lb 24D/ACRE
f're Irrig. 2 Wks

Fig. 18. Radioautograph of root of a sprayed plant. Tracer had moved to the total depth excavated following a treatment period of 2 weeks. Dosage, approximately $5 \mu\text{g}$. Exposure, 4 weeks.



Fig. 19. Plant like that of figure 16 except that the treatment period was 8 days. Note movement of the tracer into a horizontal branch (left) as well as into the vertical root.

III. UPTAKE AND DISTRIBUTION OF RADIOACTIVE 2,4-D BY BRUSH SPECIES¹

OLIVER A. LEONARD², ALDEN S. CRAFTS³

BRUSHY SPECIES of plants occupy millions of acres of land in California. Some of this land is rough, rocky, and unsuited for the growth of grass. Much of it, however, is well suited to the growth of forage species, and only the cover of brush prevents its use for grazing. Vigorous efforts are being made to reclaim this valuable land (Love and Jones, 1952) by controlled burning, reseeding, and improved range-management practices. The weak link in this program is the control of seedlings and resprouting stumps of certain brush species. Although reburning and proper grazing management are useful, chemical control is proving most valuable in this final clean-up process.

For successful chemical control of brushy plants, the chemical must penetrate the leaves or bark and move through the stem into the roots. Most resprouting occurs from a band of meristematic tissue at or somewhere below the ground line. In young seedlings this tissue is rather easily destroyed; in old plants it becomes more difficult to kill. Although 2,4-dichloro- and 2,4,5-trichlorophenoxyacetic and propionic acids are now in use, further studies of these four basic molecules must be carried out in many localities to determine the effects of differences in species, habitats, seasonal responses, and methods of application.

Obviously, a radioactive tracer that can be followed after it is applied to leaves or bark can be a valuable tool in studies on absorption and translocation. This report deals primarily with the absorption, translocation, and toxic action of 2,4-D. The carboxyl group of the 2,4-D used in these experiments was labeled with C¹⁴. This tracer, hereafter referred to as 2,4-D*,⁴ was applied to several California species of brushy plants. In conjunction with the tracer studies, branch tips were treated with nonradioactive 2,4-D to determine the extent of die-back in the various species. Two related studies were also undertaken: one a comparative analysis of the penetrative capacities of two formulations of 2,4-D*, the other a comparison between radioactive urea (urea*) and 2,4-D*.

STUDIES IN ABSORPTION AND TRANSLOCATION

Two areas representing different soil and climatic conditions were selected—one west of Davis in the foothills of the Coast Range, the other to the east in the Sierra. The growing season in the west area is early; that in the east is about a month later. The west area has rather low rainfall, the east has more. By making periodic treatments and samplings in both areas, it was possible to cover many growth stages, several species, several sites, and rather distinct differences in humidity and temperature.

Seven species were studied: blue oak, *Quercus douglasii*; wedge-leaf ceano-

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⁴ Supplied by Tracerlab.

thus, *Ceanothus cuneatus*; coyote brush, *Baccharis pilularis*; manzanita, *Arctostaphylos manzanita*; live oak, *Quercus wislizenii*; toyon, *Photinia arbutifolia*; and arroyo willow, *Salix lasiolepis*. The results with the different species are considered in the order of decreasing sensitivity to herbicidal concentrations of 2,4-D.

Treatment. Preliminary treatments started on February 17, 1953, and continued into September. The last collection of samples was taken on October 22. Each treatment involved applications to one leaf on each of three or four plants, and bark samples were collected at various distances from the treated leaf, ranging from a few inches to 4 or even 5 feet in a few cases (figs. 1, 2, 4, 5, 6, 7, and 8).

During the summer of 1954 a series of tests was undertaken in which an effort was made to apply more of the radioactive tracer. Instead of treating just one leaf, 10 leaves near the tip of a branch were treated on their under surfaces, and bark samples were taken at various levels below. These tests gave excellent radioautographs (figs. 9-14).

The treatment in 1954 was standardized at 50 μ g of 2,4-D* (1.24 millicuries per millimol) applied in a 50 per cent solution of ethyl alcohol and containing 0.1 per cent Nonic 218. Treatment consisted of applying 0.01 ml of this solution to the leaf surface and spreading it over a sufficient area that the liquid would not drip off. Applications were made to upper leaf surfaces on some plants, to lower surfaces on others. All applications were made around 10 A.M. and samples were taken one day, one week, and three weeks later from each set of treatments.

Samples consisted of bark, leaves, stem tips, and inflorescences of the treated plants. The bark was sampled by girdling the stem in two places and slipping off the ring of bark between the girdles. When the bark ceased slipping in late summer, satisfactory samples could no longer be obtained.

Radioautographing. Each bark sample was fastened flat to thick blotters by pinning it around the edges. When this preliminary drying had removed the bulk of the moisture, the samples were pressed flat between warm, dry wooden slats with C-clamps to hold them flat. Leaves, shoots, and inflorescences were dried between blotters. After complete drying, all samples from a given treatment were cemented to a piece of paper, the bark samples being oriented with the cambium side out (fig. 1). They were then placed on Eastman No-screen X-ray film and autographed for four weeks (fig. 2).

In order to obtain good contact between the samples and the film, the materials were arranged in the following order. The film was placed on a hard, flat surface ($\frac{1}{4}$ -inch plywood) and the sheet of samples was placed face down on the film. Next came a piece of heavy paper, then a $\frac{1}{4}$ -inch sheet of sponge rubber, another heavy paper, a second sheet of samples, a film, and a second plywood sheet. This sequence was repeated until a complete set of samples was ready, then the whole pile was squeezed between clamps with sufficient pressure to insure good contact between samples and films. If the materials are reversed, so that the samples are backed by the boards and the films by rubber, the samples are pressed into the film, which becomes indented, and dark pressure spots occur along all borders where the film is curved by the pressure (fig. 3).

The problems of radioautographing have still not been completely eliminated, and efforts are currently being made to find satisfactory solutions.

Means of overcoming certain difficulties were devised during the study. For example, films are spotted when strongly radioactive treated leaves are used, so treated leaves were omitted after the first samplings. The method of backing the film with a hard, smooth surface was developed after it was found that the film bent from the pressure necessary to insure close contact with the specimen. In other cases pseudoautographs were produced. Some were caused by the reaction of chemical constituents of certain species, notably willow, with the film (compare figures 2 with figures 5 and 8). These we learned to recognize by comparison with the untreated controls. Other difficulties inherent in the photographic procedure have been eliminated, and we feel that the data recorded in the tables represent a true picture of the uptake and movement of radioactive 2,4-D.

General Observations. The first autographs were the result of leaf applications made in February, 1953, when only upper surfaces were treated. These autographs suggested that the 2,4-D* was not penetrating the cuticle in leaves lacking stomata on their upper surfaces, and so all later applications that season were to the lower surfaces. Later, however, more detailed study of many autographs proved that absorption may take place from either surface. Leaves that had no stomata on the upper surface were protected against a sudden influx of high concentrations of 2,4-D*, but this protection was not afforded when the applications were made to the lower surface. Penetration is therefore likely to be rapid in leaves that have stomata on both surfaces.

The sensitivity of leaves to contact injury by 2,4-D varies with species, age of the leaves, and air temperature. Other factors influencing absorption and translocation will be considered later.

Since single leaves were treated, except as otherwise noted, the translocation of 2,4-D* was confined for the most part to a narrow strip of the bark, the portion directly connected to the treated leaves (figs. 2 and 5). When 10 leaves in a group were treated, the entire area of the bark sample produced autographs, especially within 6 inches below the lowest treated leaf (figs. 9-12); however, below the junction of the treated branches with one or more untreated branches, the radioactivity became confined to narrow portions of the bark, which appeared to be in a line of flow in the bark from the treated branch. There was some indication that 2,4-D* is less apt to be in streaks in bark above treated leaves than below them.

Branch tips treated with herbicidal concentrations of nonradioactive 2,4-D frequently died back to their connecting branches and no farther (especially noted on easily killed plants, such as coyote brush). From this reaction, plus the findings of the tracer studies, it may be inferred that if the branches are to be killed, the 2,4-D must be well distributed throughout the entire bark.

Coyote Brush

This is a moisture-loving, erect, compactly branched evergreen shrub, which grows to a height of 2 to 10 feet. It occupies vast areas in the Coast Range of California. The test plants were located in Putah Canyon in Yolo County, the inner limit of the normal range of this species. Occasional infestations occur in the Sacramento Valley and the foothills of the Sierra. The leaves are quite porous, having stomata on both the upper and lower surfaces. Some shoot growth may occur in the winter season, but the main

activity starts in April and continues into early summer, varying considerably with soil-moisture conditions. The flowering period is from August to October.

Coyote brush is successfully controlled by aircraft applications of 2,4-D (either amine or ester forms), which are generally applied from April through July, the time varying somewhat in different areas. Like certain other species, coyote brush responds much more strongly to 2,4-D than to 2,4,5-T.

Tracer Studies. There were marked seasonal differences in the absorption and translocation of 2,4-D* from treated leaves (figs. 15 A and B). In February only one third of the branches showed radioactivity outside of the treated leaves. Applications in April, at the time of vigorous new shoot development, resulted in an intense absorption and translocation of 2,4-D*. The intensity decreased slightly in May and June. No absorption or translocation was evident in July. No 2,4-D* was detectable in the bark of plants treated on a single leaf on July 31, 1953. However, treatment on August 5, 1954, when 10 leaves were treated on each branch, resulted in readable autographs as far as 12 inches from the nearest treated leaf.

The treatment time necessary to obtain the darkest autograph of the bark also appeared to vary with the season. A three-week period was apparently best in February, but one week was enough in the following months. Some 2,4-D* was present in bark one day after the applications were made, but in no case were the autographs as dark as those taken one week after treatment.

In February and March, only downward movement was evident, although in March there was also some movement into new branch tips quite close to the treated leaves. In April, on the other hand, movement was almost entirely upward; in only one case did 2,4-D* penetrate into a branch below the treated leaves; the movement upward from the treated leaves was into growing tips within 2½ inches or less from the point of application. After the month of April translocation appeared to be entirely downward.

The tests were not designed to give clear-cut information regarding the rate of translocation; however, 2,4-D* seemed to be farther down the stems one week after application than one day after. There is apparently a continuous redistribution of the radioactivity (probably 2,4-D*) within the plants. As used in these experiments, the degree of radioactivity depends upon such factors as the quantity of 2,4-D* applied and the physiological state of the plants.

Branch-Tip Treatments. Branch tips were sprayed with an emulsifiable acid formulation of 2,4-D (table 1). Appreciable die-back beyond the point of spray occurred at all times of the year, confirming field experience that coyote brush is always sensitive to spray applications with 2,4-D. However, this plant is most sensitive to sprays applied during the periods of active growth and is much harder to kill (especially by aircraft application) after the soil has become dry. The reduced die-back of branch tips in the summer accords with this observation, as well as with the reduced intensity of autographs obtained during this period.

Translocation behavior and spraying experience indicate ready absorption of 2,4-D by coyote brush leaves, active transport to the roots, and a high susceptibility to its toxic action. The periods of highest susceptibility

may reflect active root growth, which is, in turn, a response to soil moisture. When the growth is young and succulent, the amine salts are superior to the esters. Stomata on the upper surfaces of the leaves allow a rapid influx of 2,4-D into the leaves; such rapid influx of the esters brings about contact injury. The acid formulation used in these experiments resulted in some contact kill, which was evident three weeks after the applications were made; however, fewer instances of contact injury were noted on coyote brush leaves than on any other plant, except manzanita. This lack of contact injury is

TABLE 1

BRANCH-TIP TREATMENTS OF COYOTE BRUSH WITH
A 0.5 PER CENT 2,4-D ACID (EMULSIFIABLE) FORMU-
LATION IN WATER CONTAINING 0.5 PER CENT NON-
TOXIC OIL.* READINGS MADE JULY, 1954

Date of application	Percentage of tips killed by the spray	Average die-back of branch beyond the part sprayed
<i>1953</i>	<i>per cent</i>	<i>inches</i>
February 18.....	100	12
April 3.....	100	20
May 18.....	100	10
June 5.....	100	11
July 23.....	100	8
August 19.....	100	5
September 24.....	100	6
October 31.....	100	5
October 31.....	100	5

* Shell Mineral Seal Oil; viscosity 46 SUS, UR 90 per cent.

probably a factor in its successful control by aircraft applications of 2,4-D. Another factor is the excellent absorption by petioles and stems, as can be noted in figure 21.

Arroyo Willow

Arroyo willow is one of the most common willows of the valleys and foothills throughout California. Like coyote brush, it is favored by a moist environment, at least for its roots; it also responds definitely to 2,4-D treatment.

Tracer Studies. Marked seasonal differences in absorption and translocation were observed between arroyo willow, a deciduous plant, and coyote brush, an evergreen. In contrast with coyote brush, little, if any, translocation occurred in arroyo willow before April 15 (fig. 16 A, B, and C). Beginning in late April, when some leaves had reached full size, translocation of 2,4-D* appeared to take place and continued without any outstanding change throughout the summer and fall; this was associated with continuous shoot growth, which, in turn, is related to the presence of adequate soil moisture (figs. 7 and 8).

Translocation both downward and upward from the treated leaves occurred from late April until September; the date of application had little effect on either the concentration of 2,4-D* in the bark or the distance moved. However, the most intense autographs of the branch tips above the treated

leaves were made from April samples; furthermore, this was the only time that autographs were produced by branch tips from branches located below the treated leaves. Applications made in October showed no evidence of upward movement of 2,4-D*, but the movement downward was as good as or better than that during any previous month.

Seven days after treatment seemed to be the optimum time for obtaining autographs. High concentrations of 2,4-D* were not found in the willow bark, although the bark samples of other woody plants, such as toyon and

TABLE 2
BRANCH-TIP AND WHOLE-PLANT TREATMENTS OF
THE ARROYO WILLOW USING 0.5 PER CENT 2,4-D ACID
(EMULSIFIABLE) IN A WATER SOLUTION CONTAIN-
ING 0.5 PER CENT NONTOXIC OIL. READINGS
MADE DECEMBER, 1954

Date of application	Average die-back of branch tips beyond the part sprayed	Percentage kill of sprayed plants
<i>1953</i>	<i>inches</i>	<i>per cent</i>
February 17.....	0	0
March 30.....	16	0
April 23.....	50	40
May 15.....	30	80
October 21.....	46	60
November 25.....	..	0

live oak, sometimes had very high concentrations of radioactivity. Arroyo willow, however, is much easier to kill with 2,4-D than either toyon or live oak.

Branch-Tip and Whole-Plant Treatments. The results in table 2 on the die-back of branch tips treated with 2,4-D indicate that the greatest effect was produced after the leaves had become fully expanded in April and that considerable die-back occurred from all treatments, ending with that of October 21. Because the amount of die-back varies considerably among branches, seasonal changes cannot be further evaluated.

Sensitivity of the entire plant to 2,4-D parallels in a general way the responses of branch tips. 2,4-D sprays applied too soon after the leaves have first appeared in the spring result in a poor plant kill. Plant kill may not correlate very well with 2,4-D* translocation in April because the young leaves are too quickly injured by herbicidal concentrations of 2,4-D. Translocation is thus limited. In contrast, nonherbicidal concentrations, such as those used in the tracer studies, may bring about extensive translocation. This does not, however, prove that killing will be extensive.

Some contact injury resulted from the applications of 2,4-D to the lower leaf surfaces. This was evident three weeks after application in March, but in April and later months it was evident only one week after.

Wedge-Leaf Ceanothus

This plant is a common evergreen shrub of the California chaparral. Shoot growth is most active in April and May. Flowering starts in February and continues into March at the Putah Creek location. The leaves are

$\frac{1}{2}$ to 1 inch long and do not have stomata on the cutinized upper surface. The under surface has numerous stomata and is covered with microscopic hairs. Spray droplets applied to the upper surface spread rapidly over the surface, while applications made to the under surface spread very little and appear to be quickly absorbed through the stomata into the intercellular spaces. This plant does not sprout after a fire and is not considered a vigorous stem sprouter.

This species is quite susceptible to aircraft sprays, especially to applications of esters of 2,4,5-T in diesel oil; it is only slightly less sensitive to similar sprays with 2,4-D. Oil emulsions are appreciably less effective than applications in straight oil, and applications with only water as a diluent are poor. Available evidence points to a penetration problem, related either to the leaves or to the stem. The leaves are subject to contact injury when applications are made to the lower surface, but in airplane applications the spray is deposited mostly on the cutinized upper surface. This type of deposition may largely explain the plant's susceptibility to aircraft sprays, since a slow diffusion of 2,4-D through the cuticle would minimize contact kill.

Tracer Studies. Radioautograph studies yielded very little information on the absorption and translocation of 2,4-D* by this plant (fig. 17 A and B). Only the February treatments, when upper leaf surfaces were treated, resulted in positive autographs, with the exception of a single branch showing positive movement of 2,4-D* in the bark in May and the occasional appearance of 2,4-D* in leaves opposite the treated leaves. Most of the translocation in the bark was downward, although close to the treated leaves there was some movement upward, perhaps associated with the onset of flowering. The 2,4-D* was much farther down the bark one week after application than one day after.

Applications to the lower surfaces resulted in killing some of the treated leaves within one week after treatment. This may have been one factor contributing to the negative autographs obtained after February 17, the only date when applications were made to the upper surface. However, even treatment of the upper surface resulted in some spread of the solution over the surface and leaf margins, causing marginal burn one week after application.

Branch-Tip Treatments. These treatments were relatively ineffective at all times of the year, very few of the branches being killed farther than $\frac{1}{2}$ inch beyond the sprayed portion. In many cases only 25 to 50 per cent of the sprayed part (bark and wood) was killed. The poor kill of the branches with the acid formulation may have been due to poor penetration of leaves or bark. Although some delayed kill may be anticipated, this does not explain the complete and quick killing of this plant by aircraft application when esters are applied in oil (5 gallons to the acre).

Common Manzanita

This evergreen shrub flowers in midwinter; vegetative growth starts in March and is most active in April and early May. The leaves are broadly ovate; they are 1 to $1\frac{3}{4}$ inches long and $\frac{3}{4}$ to $1\frac{1}{2}$ inches wide and have stomata on both the upper and lower surfaces. Being quite pervious, either surface absorbs sprays quickly. This shrub does not sprout after a fire and

is not considered a vigorous stem sprouter. The bark is red and very thin; it peels off each year, starting in May.

Aircraft applications often produce a partial top kill, although if the plants are well covered by the sprays, complete kills result. A partial top kill the first year often becomes complete the second or third year after spraying. Delayed kill of manzanita is common, even with the sprouting species.

Tracer Studies. One day was not enough for absorption and translocation of 2,4-D* into any of the sampled parts (fig. 18 A and B). Even seven days did not seem to be enough, better autographs generally being obtained 21 days after application.

Translocation of 2,4-D* occurred in manzanita from February through May; later bark samples could not be obtained. Downward translocation was predominant, although in March and April some upward movement occurred into young leaves and stem tips close to the treated leaves. The 2,4-D* had moved farther down the stems three weeks after application than seven days after. There appears to have been a slow but continuous flow of 2,4-D* downward, indicating a gradual but continuous absorption of 2,4-D* by the treated leaves. These treatments did not cause injury to the leaves.

Branch-Tip Treatments. The die-back of manzanita branch tips beyond the sprayed portion was only 0.9 inch, even one and a half years after treatment. Death of the sprayed portions was very slow, often taking more than a year. Kill may not be complete until three or four years after treatment.

It is clear, however, that 2,4-D* moved much farther down the stems than the branch-tip treatments indicate. The tracer studies explain why delayed kill is commonly observed following aircraft applications to this plant. Manzanita leaves were the only ones that showed no evidence of contact injury from applications of 2,4-D, even though they are the most pervious of any studied and the solutions were rapidly absorbed into the intercellular spaces.

Toyon

This evergreen shrub is 6 to 10 feet high; its leaves are 2 to 4 inches long and $\frac{3}{4}$ to $1\frac{1}{2}$ inches wide. It is abundant in the lower Sierra Nevada, in the Coast Ranges, and elsewhere. Some shoot growth may occur at any time, but active growth does not start until April. Flowers develop in June or July, and the fruit matures about Christmas time, hence its common name, Christmas berry.

There are no stomata on the heavily cutinized upper leaf surface. The lower surface is pervious to applications of oil, the mesophyll appearing oil-soaked within a few minutes after the application of an oil droplet. No penetration is visible from similar applications made to the upper surface.

Toyon is only moderately sensitive to broadcast applications of 2,4-D. One- and two-year sprouts (developing after a fire), however, appear to be quite easily killed, especially when the sprays are applied from February to mid-April; effectiveness seems to decrease gradually after this time.

Tracer Studies. Autographs made seven days after application were generally stronger than those made one day after (fig. 19 A, B, and C). Excellent

autographs were still obtained 21 days after treatment. The most intense autographs were produced by applications made in April, May, and June (figs. 2, 5, and 8), while the faintest were those from the September and October treatments.

Movement appeared to be primarily downward in February and March, but 2,4-D* appeared in the young tips and leaves directly above the treated leaves from April through July (fig. 8). Autographs obtained three weeks after the March 17 application gave the only evidence of radioactivity in side branches below the treated leaves. This result indicates a shift in the direction of translocation during the three-week period that coincided with the beginning of active shoot growth in the spring. Translocation appeared to be entirely downward in the fall, but the total radioactivity in the bark was less than at previous times.

TABLE 3

BRANCH-TIP TREATMENTS OF TOYON AND LIVE OAK
WITH 0.5 PER CENT 2,4-D ACID (EMULSIFIABLE) IN
WATER CONTAINING 0.5 PER CENT NONTOXIC OIL.
READINGS MADE JULY, 1954

Date of application	Average die-back of toyon branch tips beyond sprayed part	Average die-back of live oak branch tips beyond sprayed part
<i>1953</i>	<i>inches</i>	<i>inches</i>
February 17.....	7	3
March 30.....	8	0
April 23.....	10	2
May 15.....	3	3
June 17.....	1	3
July 16.....	1	0

Branch-Tip Treatments. The average kill of branch tips beyond the part actually sprayed was never very great (table 3). This suggests one reason why toyon is readily killed by 2,4-D sprays only when the sprouts are sprayed within one or two years after a burn. Leaves are quite close to the roots at this time so that translocation of lethal concentrations for only a few inches may be enough to kill the underground parts that have dormant buds. The die-back of branches beyond the sprayed portion was greatest in the winter and early spring and then decreased.

The treated area of toyon leaves turned red 21 days after treatment in April. Dead spots were present seven days after applications in June and July. It is clear that toyon leaves can suffer contact injury from 2,4-D and that this factor is more important in late spring and summer than in winter and early spring.

It should be emphasized that branch-tip treatments may require more than a year to be effective. Hence, some delayed kill may occur, which is quite common on toyon sprayed with 2,4-D. It should also be emphasized that the ultimate distribution of 2,4-D* within the plant cannot be determined within 7 or even 21 days after application.

Blue Oak

Blue oak is a deciduous tree common in the foothills of the Sierra Nevada and in the Coast Ranges. The best kill of this species by aircraft application was 25 per cent with a single application (Leonard and Harvey, 1956). Sprouts are susceptible to ground applications with 2,4-D amines or esters applied in May. Reaction to the 2,4,5-T esters appears to be poor at this time of the year; however, in late June or July 2,4,5-T esters are much more effective than when applied earlier, and 2,4-D is less so. These differences are probably related to at least two factors: (1) 2,4,5-T is a better penetrating agent than 2,4-D; and (2) contact injury to leaves decreases with age or maturity.

Tracer Studies. Tracer studies were negative for the most part. Sampling started March 24 and continued until June 23, when all of the season's leaves were fully expanded; shoot growth had ceased and the bark no longer slipped. Out of 42 samples autographed—including tests run one day, one week, and three weeks after application—only five showed any movement of 2,4-D* out of the treated leaf, and the average distance in which discernible autographs were produced was 5.2 inches. Two of these were three-week samples treated in April and collected April 28. Three were one-week samples treated May 19 and May 26 (approximately the optimum season for treatment with 2,4-D).

Blue oak leaves are quite sensitive to the contact action of 2,4-D, whether in acid, amine, or ester form, and the small quantities of 2,4-D* applied to leaves in the above tests frequently killed them. It seems entirely possible that this is the principal reason for the limited translocation that was observed.

These findings corroborate the experience that spray treatment on blue oaks seldom results in more than local response. Small trees may occasionally die, but in large ones movement seldom extends beyond the main trunk. In the season of treatment, after the sprayed leaves have died, dormant buds along the branches may develop, and their leaves may be large and misshapen. Also, ridges of callus may form beneath the bark, and the smaller branches are often killed. Usually, however, the trees recover during the year following treatment. Blue oak is one of the species least responsive to aircraft spray treatment with the phenoxyacetic acid herbicides.

Live Oak

This species is an evergreen tree with oblong leaves 1 to 2½ inches long and ¾ to 1½ inches wide. The glabrous upper surface is well cutinized and contains no stomata; the under surface is not heavily cutinized and has numerous stomata.

Live oak sprouts vigorously after a fire or after being cut. Aircraft application has not yet killed a single tree; consequently, most attention has been given to the reaction of sprouts to complete coverage with foliage sprays. Sprouts have been killed by one or two applications of a mixture of 2,4-D and 2,4,5-T esters in 1 per cent diesel oil emulsion applied in May or June. Trees, also, can be killed by placing the amine salt of 2,4-D in a ring of deep cuts around the bottom of the stems.

Tracer Studies. Autographs obtained one week after application were

generally more intense than one day after (fig. 20 A, B, and C). From February to May, autographs were as intense three weeks after application as one week after; however, in May and later, the one-week period was the most satisfactory.

Translocation was entirely downward in February, but there were traces of 2,4-D* in new leaves, bark, and stem tips above the treated leaves after growth started in March (fig. 5), and there were occasional traces of 2,4-D* above the treated leaves even in July; however, 2,4-D* movement upward was never very intense. The detectable distance of movement of 2,4-D* was not great in any case, usually less than 13 inches; the greatest movement seemed to occur from February through May.

Branch-Tip Treatments. As may be seen in table 3, the kill of live oak stems beyond the sprayed portion was slight. The 2,4-D* studies show that the chemical moves in rather high concentrations for at least 6 inches beyond the point of application, and it seems likely that fairly high concentrations may at times be present at least a foot or more down the stems from the treated leaves. The actual kill of live oak by 2,4-D is extremely slow, however, and may not take place for two and a half years or more after treatment. In many cases delayed kill of live oak has followed application of the amine salt formulations of 2,4-D (Emrick and Leonard, 1954).

Live oak leaves appeared to be uninjured by the contact action of 2,4-D until May 14, when dead areas were produced within seven days after treatment; some injury resulted from treatments applied in July. Young leaves are more apt to be injured by the contact action of 2,4-D applied to the under surface than are old leaves. This may be related to the more extensive and thicker cuticle that is present on the undersides of old live oak leaves.

PENETRATION STUDIES

Two different solutions were used in studies on penetration. One solution (A) consisted of 0.5 per cent 2,4-D* acid, plus 1 per cent Vatsol OT (dioctyl ester of sodium sulphosuccinate, an anionic surface active agent), plus about 1 per cent Amine 220 (1-hydroxyethyl-2-heptadecenyglyoxalidine, a cationic surface active agent), plus 2 per cent of a paraffinic oil (viscosity 46 SUS and a UR of 90 per cent), plus water. The second solution (B) consisted of 0.5 per cent 2,4-D* acid, plus 45 per cent of tetraethyl orthosilicate, plus 45 per cent of a dimethyl silicone having a viscosity of 40 centistokes. Leaf treatment clearly showed solution B to be much more penetrating than solution A.

An 0.01-ml droplet of each of these solutions (50 μ g of 2,4-D* acid) was applied to the upper and lower surfaces and to the petioles of young and old toyon and live oak leaves and to young coyote brush leaves. Samples were collected one week after the applications were made. The autographed results are shown in figure 21 A, B, and C.

Most solutions of 2,4-D result in some contact injury to leaves, regardless of formulation; however, solution A was slightly more toxic than the standard 2,4-D* formulation because it included Vatsol OT, which has appreciable contact toxicity. Solution B, containing tetraethyl orthosilicate, was considerably more toxic than A; the dimethyl silicones appear to have no contact toxicity. The results, especially with solution B, should be considered

in the light of the above-mentioned toxicity. The spreading and penetrating properties of solution B were unusually good, and it appeared to be better in this respect than any of the oils with which it was compared (for example, n-tetradecane, n-decane, n-dodecane).

Applications to the upper surfaces of live oak and toyon leaves gave no visible evidence of penetration during the one-week experimental period; these observations were in contrast to the rapid penetration of the mesophyll when applications were made to the lower surfaces. With coyote brush leaves, the solutions penetrated rapidly when applied to either upper or lower surfaces. Petiole applications were not confined to the petioles; there was some rundown into the axillary buds.

Results with young toyon leaves indicate that with solution A there was little difference in the absorption and translocation of 2,4-D* whether upper or lower surfaces were treated; however, with solution B (silicone) the upper-surface application resulted in effective absorption and translocation of 2,4-D*, while applications to the lower surface were completely ineffective. The negative results from treating young toyon leaves with solution B were anticipated and were due to the sensitivity of the young tissues to contact injury by the tetraethyl orthosilicate.

Old toyon leaves responded somewhat differently than the young leaves. This may be because of the increased thickness of the cuticle (reduced cuticle penetration) and because of an increased resistance of the older tissues to contact injury. When solution A was applied to the upper surface of old toyon leaves there was no detectable penetration of the cuticle by 2,4-D* within the experimental period, but good autographs were produced from applications made to the lower surfaces. In contrast, applications of solution B to either upper or lower leaf surfaces resulted in good autographs; solution B was evidently not toxic enough to the tissues of mature toyon leaves to prevent translocation from applications made to the lower surfaces.

Live oak leaf responses differed slightly from those of toyon. The differences can be interpreted as indicating that live oak leaves are more sensitive to contact injury than toyon leaves. Other lines of evidence also support this belief.

Young live oak leaves, like young toyon leaves, absorbed and translocated solution A to about the same degree from upper- or lower-surface applications; however, solution B, when applied to the upper surface, resulted in good autographs, but when applications were made to the lower surface, the autographs, if any, were faint. Results with old live oak leaves followed the same general pattern as that obtained with the young leaves; however, with solution A absorption was poor, while solution B resulted in excellent absorption, especially when applied to the upper surfaces.

With coyote brush the responses were similar. Absorption and translocation were excellent from solution A applied to either surface, but very little took place with solution B. In the case of solution B, contact injury prevented the leaves from functioning.

Petioles usually responded rather well to applications of either solution A or B; however, for the most part, the strongest autographs were obtained with solution B. Actually, petiole applications represent applications to the axillary buds and to the stems, and most of the absorption probably takes

place here. This is especially true with coyote brush, which has practically no true petiole; the droplets applied near the leaf base were observed to spread to the buds and up and down the stems for perhaps an inch.

TRANSLOCATION OF UREA* AND 2,4-D*

Radioactive urea and radioactive 2,4-D were made to the same specific activity in 50 per cent alcohol. The purpose was to compare these two different chemicals as to penetration and translocation. It is well known that when urea enters the leaf cells, it is decomposed to ammonia and CO_2 by the enzyme urease (Kuykendall and Wallace, 1954; Sumner and Myrback, 1951). Most of the radioactive carbon dioxide is possibly converted into sucrose and translocated in this form.

A 0.01-ml droplet of solution was applied to the underside of each of 10 adjacent leaves near the ends of the branches of toyon, live oak, and coyote brush. One week later the bark was sampled as previously described and autographed for 30 days. On each plant, one branch was treated with 2,4-D* and another with urea.*

Data from these tests are presented in figures 10 and 22. The 2,4-D* appeared to be absorbed slightly better than the urea* by all three plants. There was no appreciable difference in the translocation of either the 2,4-D* or the urea*; however, there is some suggestion that in live oak urea* moves more freely than 2,4-D*, which may indicate some chemical binding of the 2,4-D either in the sieve tubes or in other cells. Such binding has been indicated as possible in some plants with MCP (2-methyl-4-chlorophenoxyacetic acid), according to Brian and Rideal (1952).

The radioactivity was, for the most part, well distributed throughout the bark within 6 inches of the treated leaves, but at greater distances it was commonly restricted to certain parts of the bark. A uniform distribution in the bark near the treated leaves would be expected; as the treated branch joined other branches, the radioactivity would automatically be confined largely to those portions of the bark directly connected to the treated branch. This is apparently what occurred. However, the distribution in the bark was not always confined to streaks, nor was it always present in general or uniform concentrations. Thus it is clear that there is some lateral migration and utilization of the 2,4-D* or urea*, probably as a result of intense cambial activity in the shoot. This may be one explanation for the decrease in concentration of radioactivity, even within the "streaks" or the limited portions of the bark responsible for translocation. The urea* tended to be more generally distributed in the bark than the 2,4-D*, indicating a greater lability of urea or products derived from its radiocarbon, such as sucrose.

DISCUSSION AND CONCLUSIONS

A number of points of interest have arisen since the last report on this project (Crafts and Stewart, 1954). The early autographs indicated that little 2,4-D* was penetrating the cuticle on the upper sides of hypostomatal leaves, and subsequent treatments were therefore confined to the lower surfaces. Careful study of the original autographs and a number of later ones, however, showed that the tracer will penetrate the cuticle in the absence of stomata. In the penetration studies it was apparent that the

intact cuticle may protect the mesophyll from too rapid absorption of the toxicant, which would result in rapid injury and an inhibition of translocation. This effect may explain the excellent results of airplane application of an oil formulation of 2,4-D ester on wedge-leaf ceanothus (Leonard and Harvey, 1956), whereas a similar treatment on young leaves of blue oak fails because of the contact injury to the leaves.

This finding re-emphasizes the significance of the whole problem of the penetration of herbicides into leaves and the relation of contact toxicity to uptake and transport. Possibly many of our present failures result not from lack of absorption but rather from too rapid and great an uptake, resulting in injury and failure to translocate.

The relation of 2,4-D transport to food movement in the plant is evident throughout this work. Also important is the fact, borne out by much experimentation, that only growing cells and tissues respond to 2,4-D. These facts emphasize the importance of thoroughly understanding the physiology of the plants to be treated and the difficulties of meeting all the requirements for success.

Translocation takes place throughout a fairly long portion of the year, particularly in evergreen species. As long as the plants are synthesizing foods and using them in growth, flowering, fruiting, and storage, transport is possible within the plant. Failure to kill active plants with green foliage apparently seldom results from the toxicant's failure to translocate. It may, rather, result from lack of absorption, from translocation to the wrong tissues (flowers, fruits, or vegetative shoots), or from the roots' inability to respond. Because root response to 2,4-D involves active root growth, available soil moisture is essential to successful treatment. Inadequate soil moisture is one of the greatest obstacles to successful use of hormone herbicides in brush control in the West.

The period for successful treatment of many brushy species is apparently a relatively brief time in the spring or early summer when the leaves have fully expanded but have not become too heavily cutinized. If chemicals are to be effective, soil moisture must still be available for root growth. Weather conditions, too, should be favorable for treatment (moderate temperature and a humidity above 20 per cent). In seasons of low rainfall such periods may last only a few days; in extreme cases there may be no such time at all. This has been the case with respect to mesquite control in Texas and Arizona during a succession of dry years.

Continued spray programs on many species have proved that, in contrast to blue oak, arroyo willow, which thrives only in moist habitats, may be treated throughout a rather long period from the time the leaves have fully expanded until late in the autumn (Leonard and Harvey, 1956). And, in contrast to these two deciduous species, several evergreen species may be treated in late winter or early spring while the previous season's leaves are still green and active and during the rainy season when soil moisture is amply available. Toyon and wedge-leaf ceanothus are such species.

It would be wrong to imply that absorption, translocation, and root activity are the only factors involved in successful treatment of brushy plants with 2,4-D. Species susceptibility, temperature, nutrition, secondary invasion by fungi and bacteria, and many other factors are undoubtedly important. But, for successful results, at least four physiological factors

must be favorable: (1) The herbicide must be absorbed. (2) Photosynthesis must provide assimilates for movement through the phloem. (3) Translocation from active, photosynthesizing leaves to the roots must be going on. (4) Root activity must involve meristematic activity and growth, not just storage.

In California these four functions may not coincide in a number of brushy species within a given locality. For example, manzanita, wedge-leaf ceanothus, toyon, live oak, and coyote brush are perennials with green leaves throughout the winter. Arroyo willow, blue oak, and poison oak, on the other hand, are deciduous, and the time between leaf maturation and the depletion of soil moisture may be short.

Blossoming in the seven species used in this study may occur as early as January (manzanita) or as late as July or August (coyote brush). Hence, budding or blossoming cannot be used as a criterion for determining time of treatment as it can in many herbaceous perennials (Crafts, 1956). A further consideration involves the stage of growth and the conditions of growth in the brushy plants. Oaks, for instance, may be treated as young seedlings in their first year's growth, as small trees or shrubs, or as old, mature trees with trunks several feet in diameter. The method of treatment varies, of course, according to each stage of growth. Brush seedlings may be growing in competition with forage species such as grasses and clover, or they may be in competition with themselves and other brush species in dense stands of brush. The availability of soil moisture, and hence the optimum time for treatment, may vary widely under these different conditions. Obviously, there can be no optimum time for treating mixed stands of brush under these western range conditions, and even in pure stands conditions may be such that the optimum time may vary according to the age and nature of the plant stand. Years of research will be required to study all the variables concerned, and radioactive isotopes will be valuable tools.

It seems quite obvious that fire will be the primary tool for brush clearance on ranges in many western states. Chemicals will provide a supplementary, but an essential, method for eliminating seedlings and killing stumps of resprouting species. They are a primary tool in treating trees in woodlands where fire, for many reasons, is undesirable.

Where fire is used, reseeding with tested forage varieties is essential (Love and Jones, 1952), and management must be aimed at the establishment and maintenance of successful stands of forage. Reburning may be used in many places to control brush seedlings and to suppress resprouts. However, after the initial burning and seeding, chemicals provide a valuable method for eliminating seedlings and resprouts. Chemical control of brush may provide the best and most useful method in many situations on almost every range.

SUMMARY

After suitable methods had been devised, studies on the absorption and translocation of radioactive 2,4-D (2,4-D*) were carried out on seven species of woody plants common to California.

Coyote brush (*Baccharis pilularis*): 2,4-D* was absorbed and translocated slightly in February, intensely in April, less intensely in May and

June, and not at all in July. In February and March movement was downward from treated leaves, in April almost entirely upward.

Arroyo willow (*Salix lasiolepis*): Little translocation of 2,4-D* occurred before April 15; from late April until late summer translocation of the tracer was continuous. Translocation both upward and downward from treated leaves occurred from late April until September. Upward movement was predominant in April; in October all movement was downward.

Wedge-leaf ceanothus (*Ceanothus cuneatus*): little translocation of 2,4-D* was observed. Application to the lower surfaces of leaves resulted in rapid killing of the treated leaves; this may explain the failure of uptake and transport of the tracer.

Manzanita (*Arctostaphylos manzanita*): 2,4-D* was absorbed and translocated throughout May; after this time bark samples could not be obtained. Some upward movement took place in March and April; in general, downward translocation was predominant.

Toyon (*Photinia arbutifolia*): Absorption and translocation of 2,4-D* were prominent from February through October; transport was primarily downward in February and March, upward in midspring and summer, and downward again by fall. Radioautographs obtained from treatments in September and October were faint.

Blue oak (*Quercus douglasii*): Tracer studies were mostly negative. Starting in March and continuing until June 23, only 5 out of 42 samples showed any movement, and the average distance of transport was only 5.2 inches. Blue oak leaves are very sensitive to contact action by 2,4-D. This probably explains the poor results of the tracer tests and the similar poor response of blue oak trees in the field to 2,4-D treatment.

Live oak (*Quercus wislizenii*): Translocation of 2,4-D* was active from February through September. Movement was entirely downward in February, somewhat upward after new growth started in March, but largely downward through the rest of the season.

These studies emphasize the deleterious effect of contact injury on the uptake and transport of this herbicide. They strengthen the evidence for a correlation between food movement and 2,4-D movement in plants. They show that the chemical may move in evergreen species for many months, whereas in deciduous species it may move only during relatively short periods. They pinpoint the importance of soil moisture and root growth to 2,4-D transport and response. And finally, they prove that different species require different treatment and that a single application cannot be expected to control mixed brush populations under California conditions.

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Fig. 1. Samples of bark, stems, and leaves of toyon, arroyo willow, and live oak cemented to a sheet of paper and ready to be placed, face down, on the X-ray film.

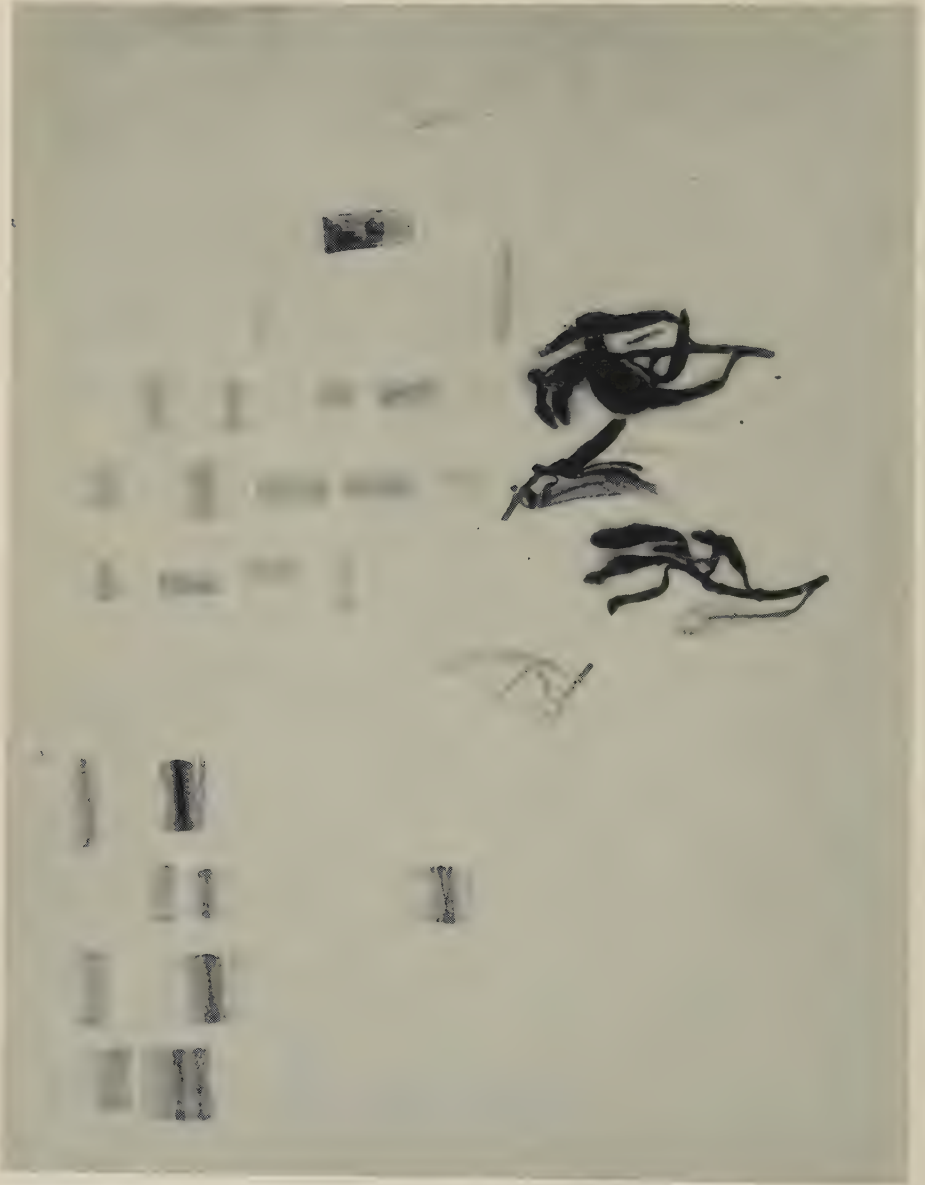


Fig. 2. Radioautograph of the plant samples shown in figure 1. The plants were treated on April 15. Samples were taken April 24. Exposure on the film was four weeks. Movement has been upward and downward in willow, downward in toyon and live oak.

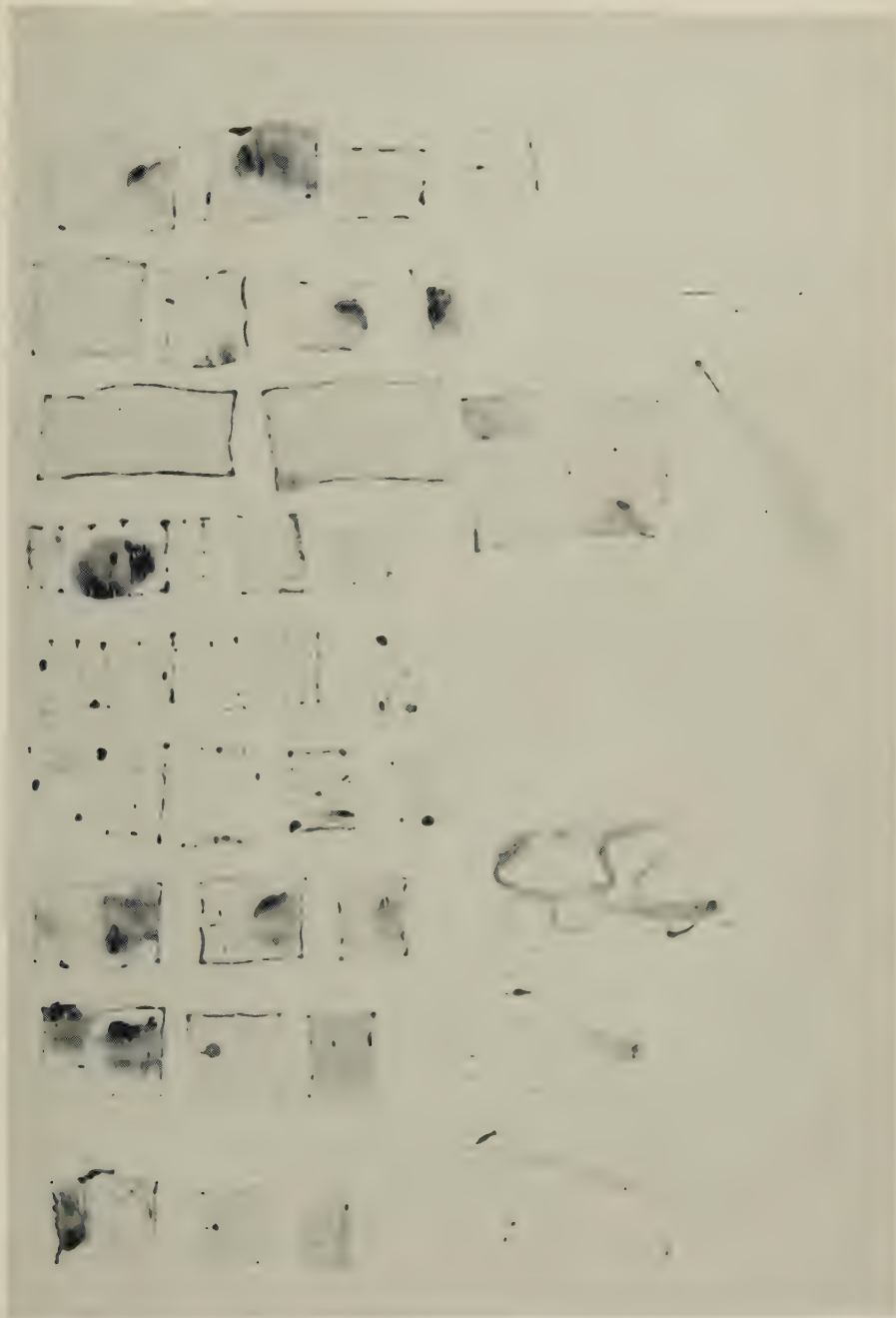


Fig. 3. Radioautograph made with high pressure and soft backing for the film.
Compare with figure 2, obtained with less pressure and a firm backing.



Fig. 1. Samples of live oak, arroyo willow, and toyon from plants treated May 14 and sampled May 21.

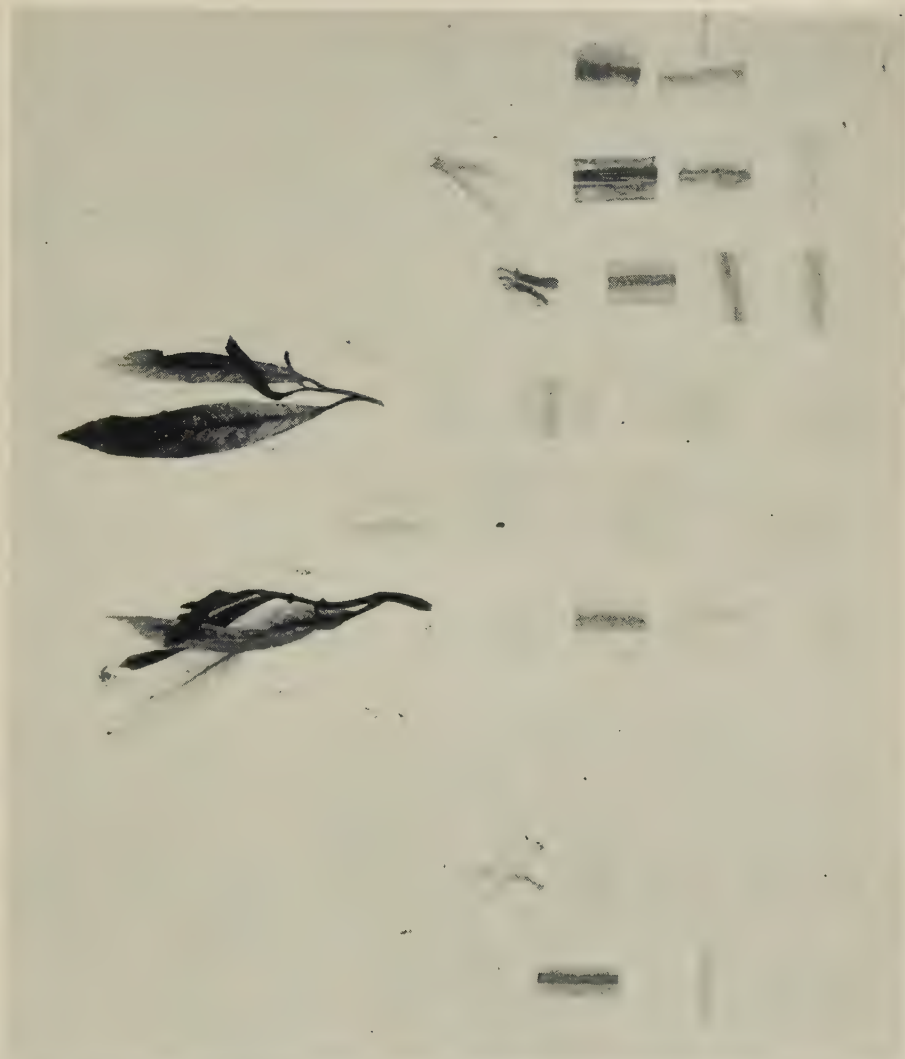


Fig. 5. Radioautograph of the samples of figure 4. Note that movement has been upward in the two willow tips sampled and upward in two of the three toyon shoots and one (middle) of the live oak shoots. All three show some downward movement.



Fig. 6. Radioautograph of one of the first collections. The species are toyon, arroyo willow, and live oak, and the treated leaves as well as bark are shown. These treated leaves were so high in radioactivity that they caused spots on other films in the bundle being exposed. In subsequent samplings, treated leaves were omitted.



Fig. 7. Samples of live oak, arroyo willow, and toyon from plants treated May 14 and sampled June 4.

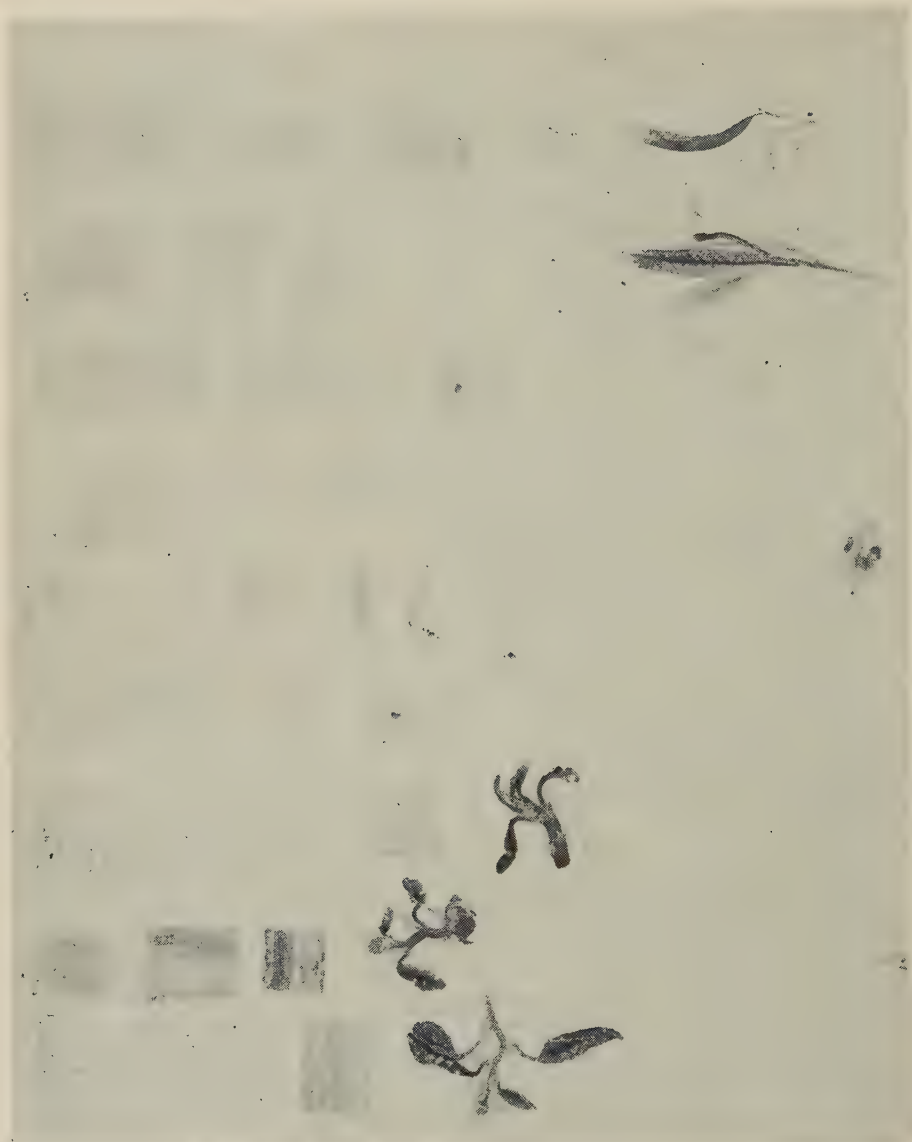


Fig. 8. Radioautograph of the samples of figure 7. Movement after three weeks in this willow plant has been slight and in both directions; in live oak it has been downward only; in toyon some downward movement has occurred, but it is strong in an upward direction into the inflorescences. Live oak at this time had formed terminal buds and there was no shoot growth.



Fig. 9. Samples of live oak, toyon, and coyote brush plants treated April 5, 1954, and sampled April 13, 1954. The plants received two radio-active tracers, ^{24}P and urea*. Ten leaves near the tips of branches were treated, each receiving 0.01 ml of a 50 per cent alcohol solution containing the isotope at a concentration that gave 400 counts per minute when 0.01 ml was dried on a planchet 26 mm in diameter. The two top bark samples were taken within the treated region on the shoot. The third sample was taken 1 to 2 inches below the treated region; the fourth sample, 3 to 4 inches below; the fifth sample 6 to 7 inches below; the sixth sample, 12 to 13 inches below; the seventh sample, 24 to 25 inches below; and the eighth sample, 36 to 37 inches below. The first vertical row in each group received ^{24}P , the second area*, the third $^{24}\text{D}^*$, the fourth urea*, and so on. Row 9 is a control.



Fig. 10. Radioautographs of samples shown in figure 9.



Fig. 11. Bark and shoot samples of live oak and toyon plants treated June 1, 1954, and sampled June 8. The plants received 2,4-D* at the same rate as in figure 9. Shoot tips shown were taken immediately above the treated region. Growth at the tips of these plants was slowing down, and the top 10 fully expanded leaves were treated. The first, third, and fifth vertical rows represent the treated samples, rows 2 and 4 are controls. There was no upward movement in live oak but strong upward movement in toyon. Downward movement was pronounced in one shoot of each species but not extensive. Note the streaking in the downward movement.



Fig. 12. Radioautographs of samples shown in figure 11.

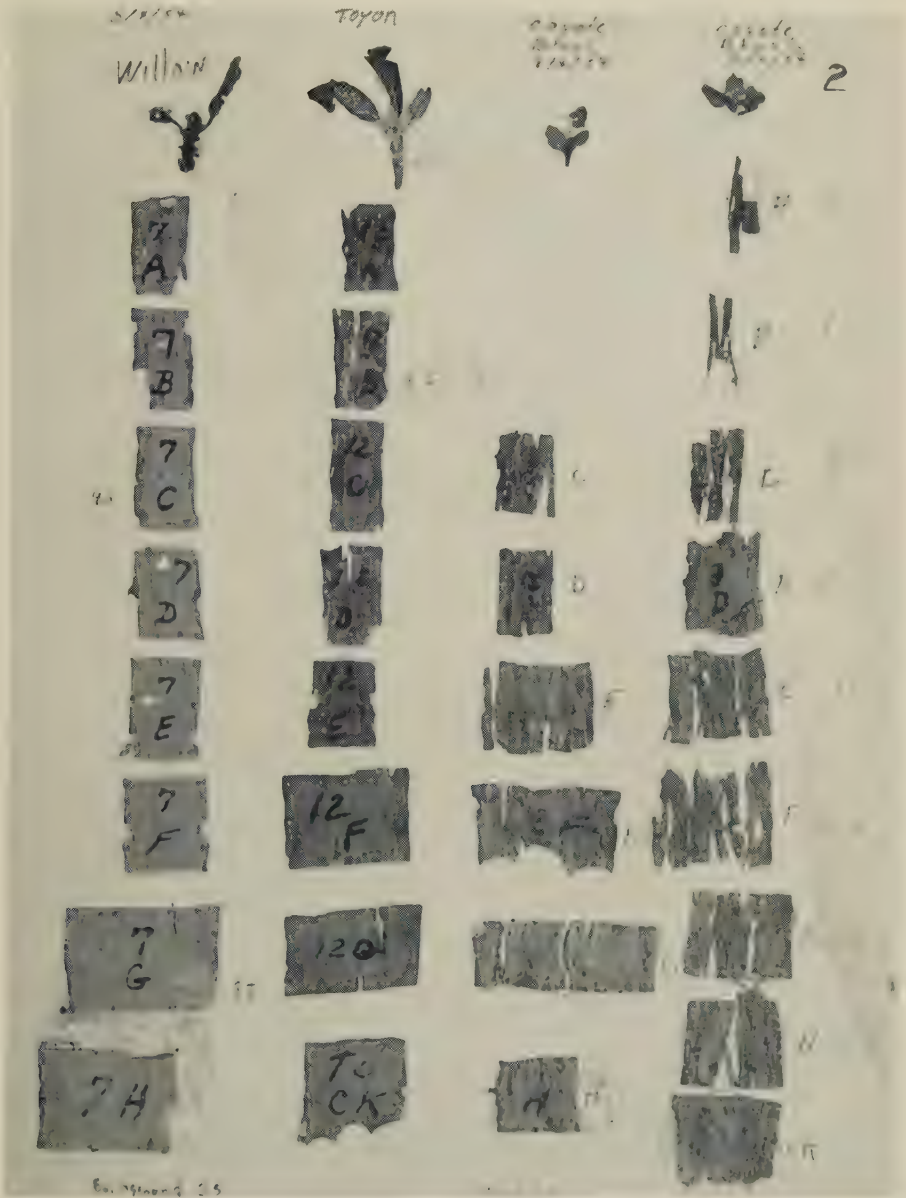


Fig. 13. Bark and shoot samples of arroyo willow, toyon, and coyote brush plants treated August 4, 1954, and sampled August 15. Treatments were as in figure 10, and sampling was the same. There was both upward and downward movement in willow and toyon, but only downward movement in coyote brush. Bark of the latter was starting to stick and hence difficult to sample. A few counts were made on these samples. On willow, sample C had a count of 40, G a count of 37, and both H and the background a count of 35. On toyon, sample B had a count of 83.

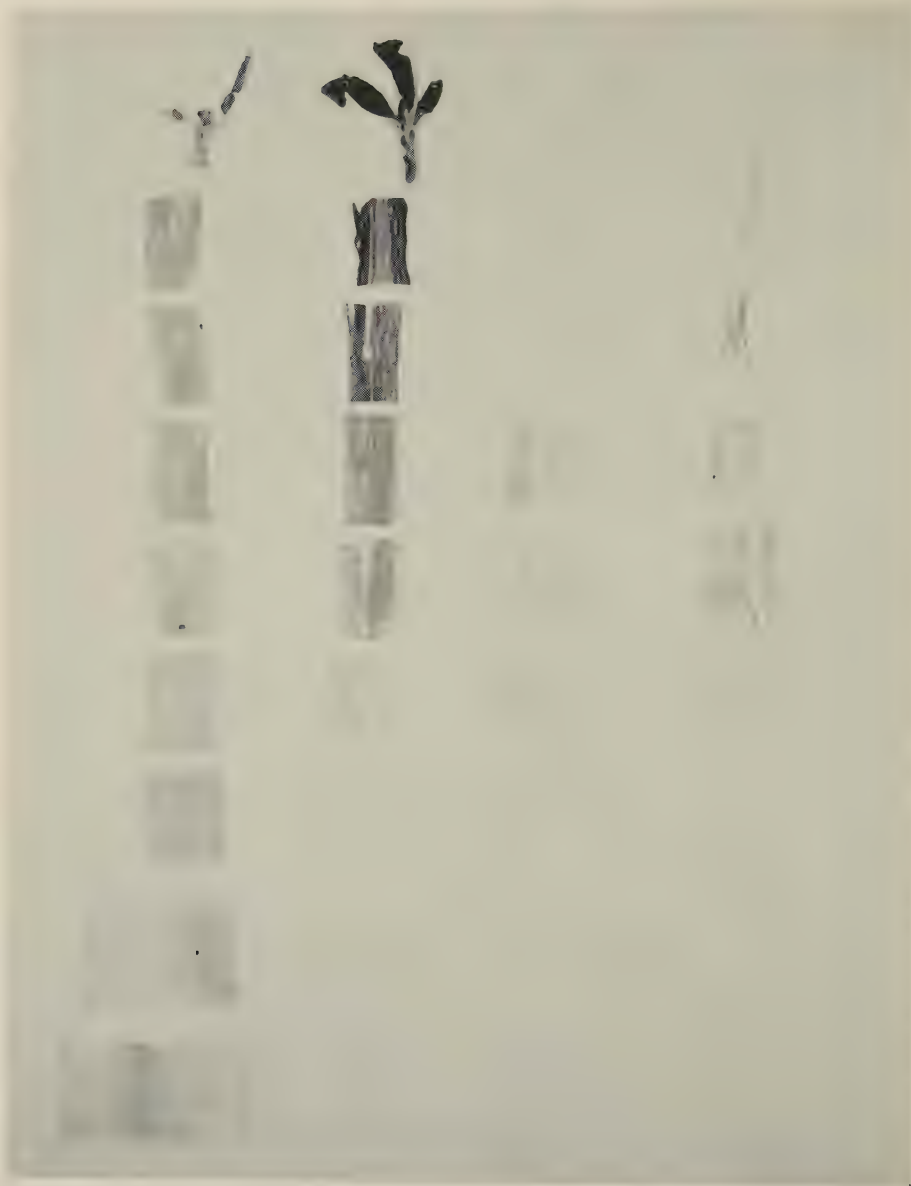


Fig. 14. Radioautographs of samples shown in figure 13.

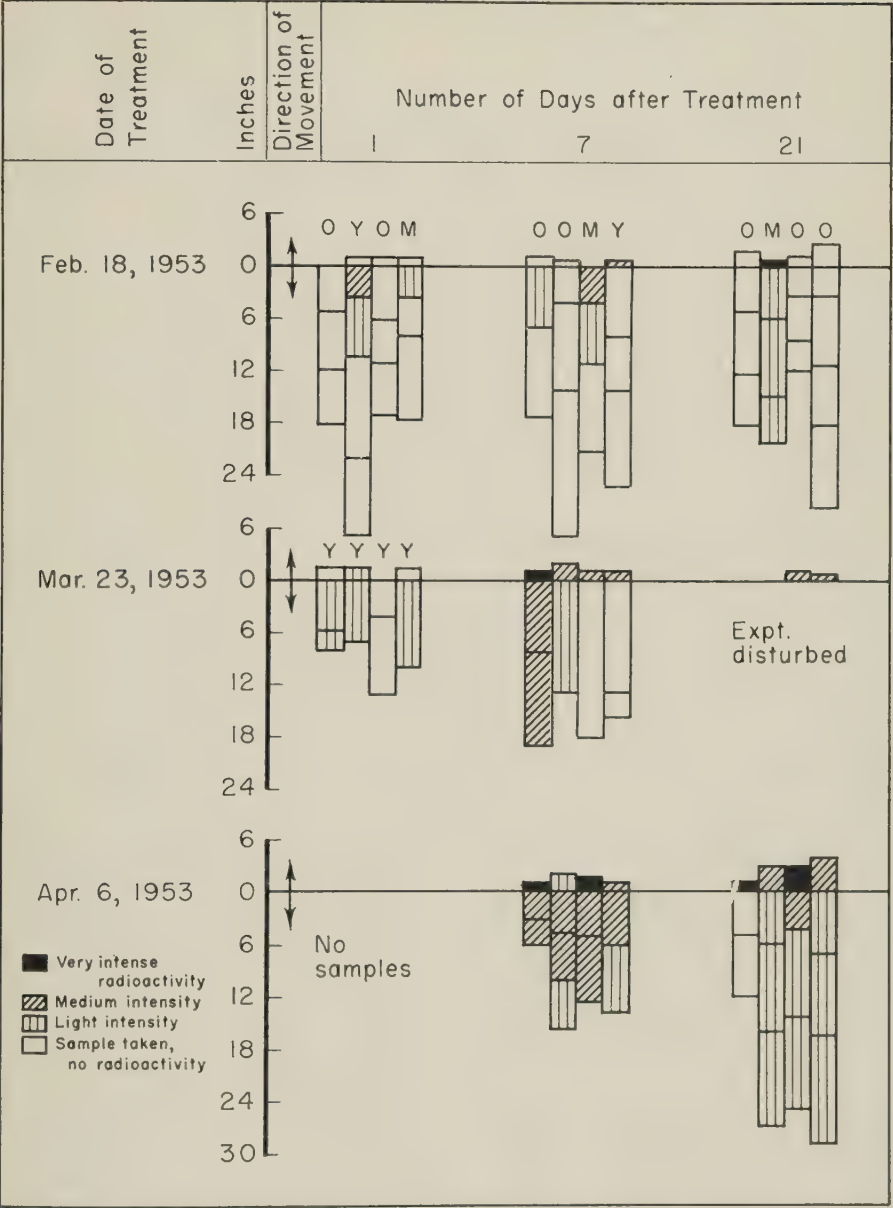


Fig. 15A. Charts showing distribution of radioactivity in shoots of coyote brush after leaves were treated with 2,4-D*. Bars above zero lines represent upward movement of the tracer; below zero, downward movement. O = old leaves; M = mature; Y = young.

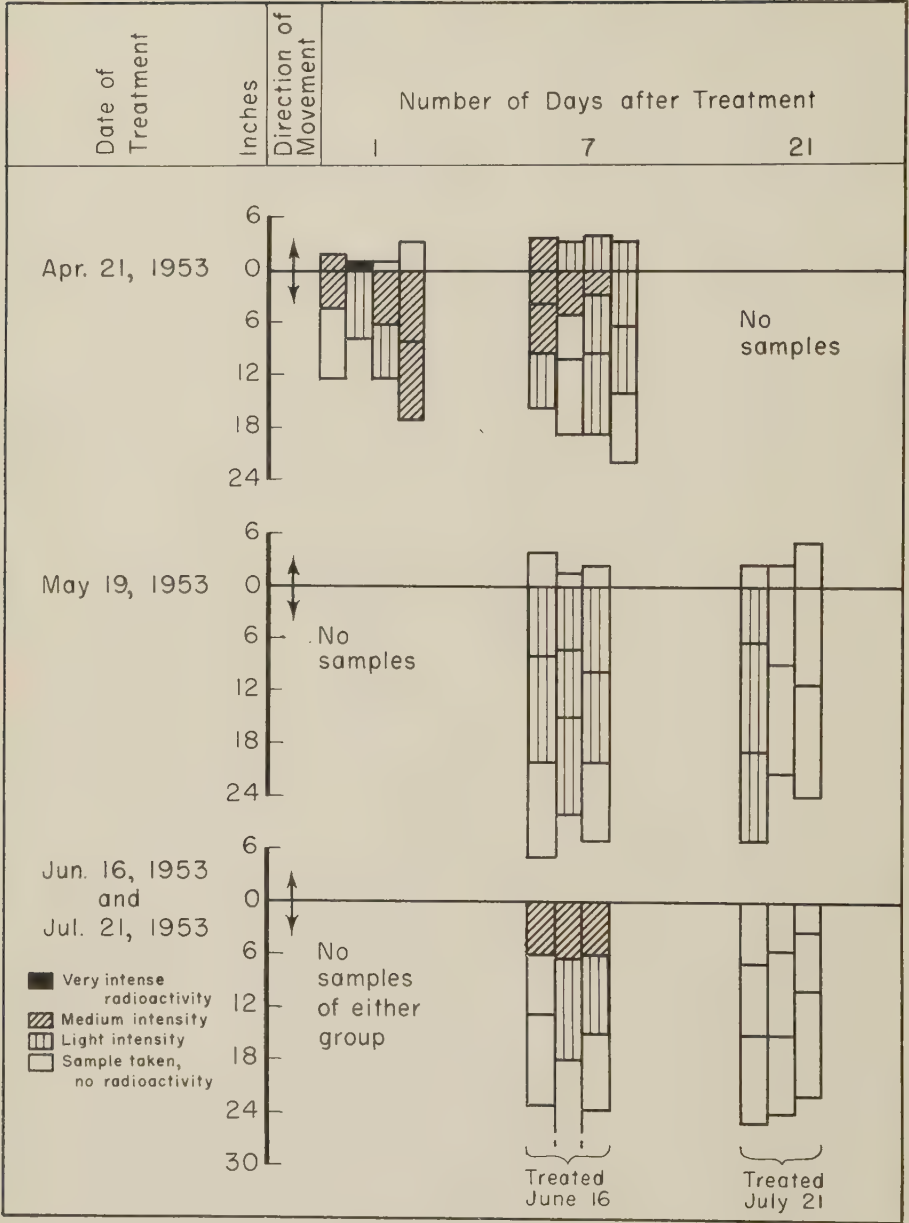


Fig. 15B. Coyote brush: same as 15A except that all treated leaves were mature.

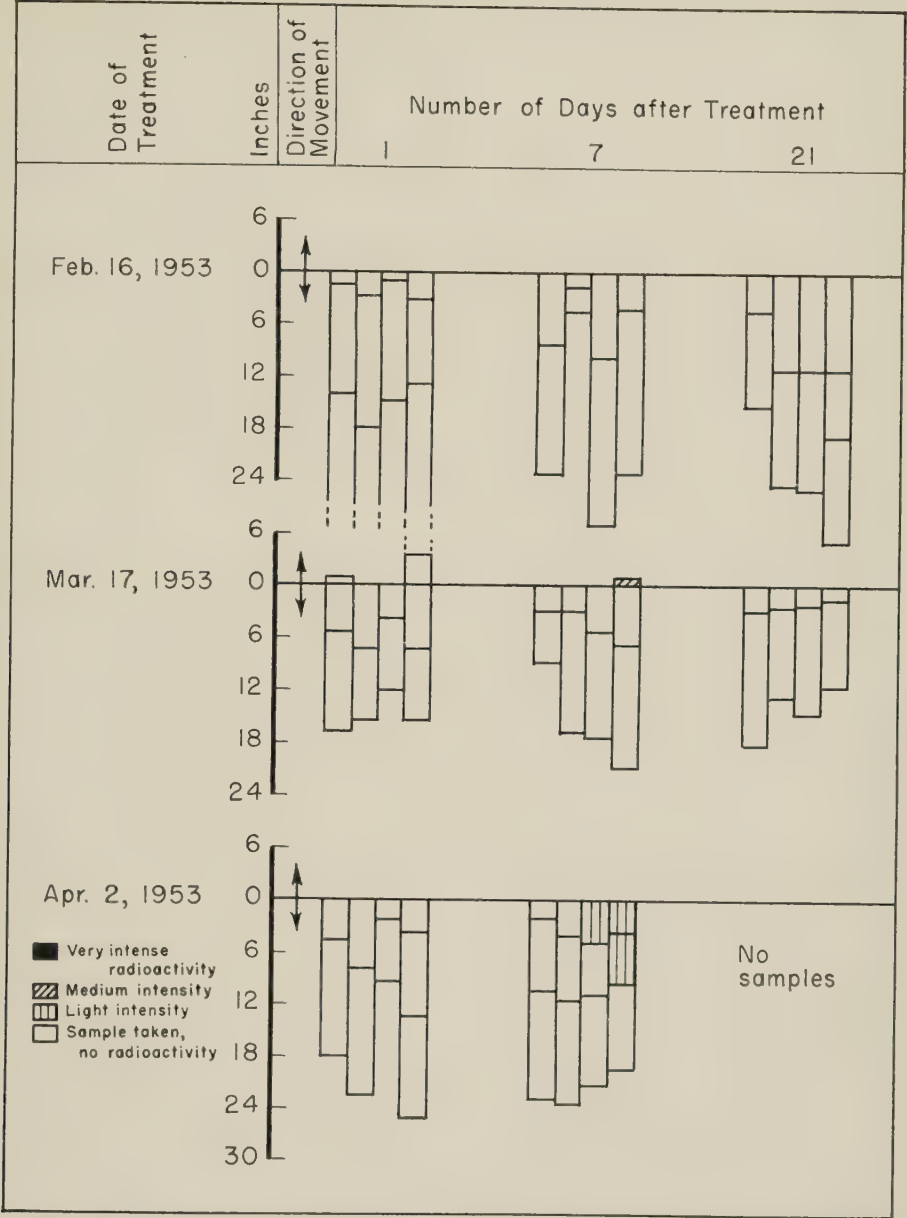


Fig. 16A. Charts showing distribution of radioactivity in shoots of willow after leaves were treated with 2,4-D*. Bars above zero lines represent upward movement of the tracer; below zero, downward movement. All treated leaves were young.

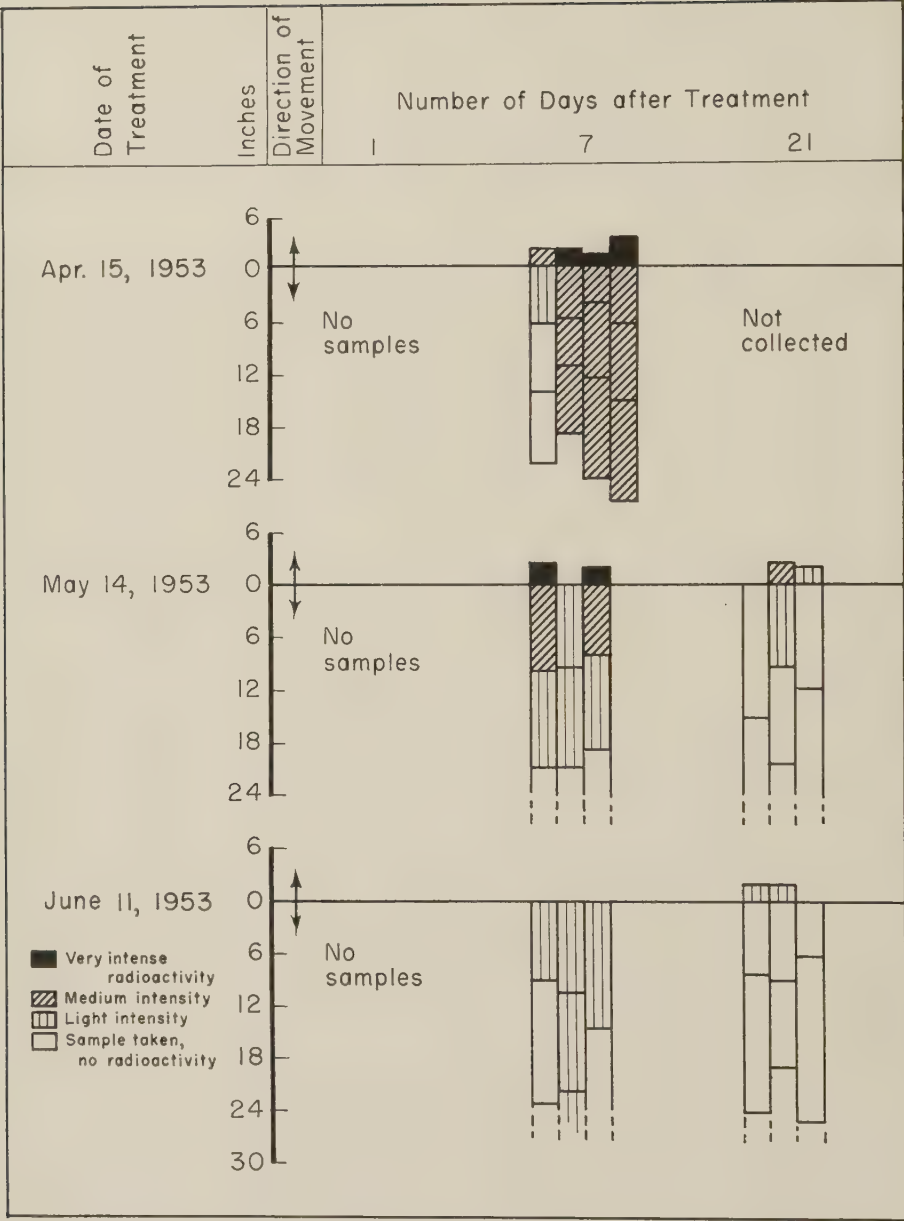


Fig. 16B. Willow: same as 16A except that treatments were later as indicated. All treated leaves were mature.

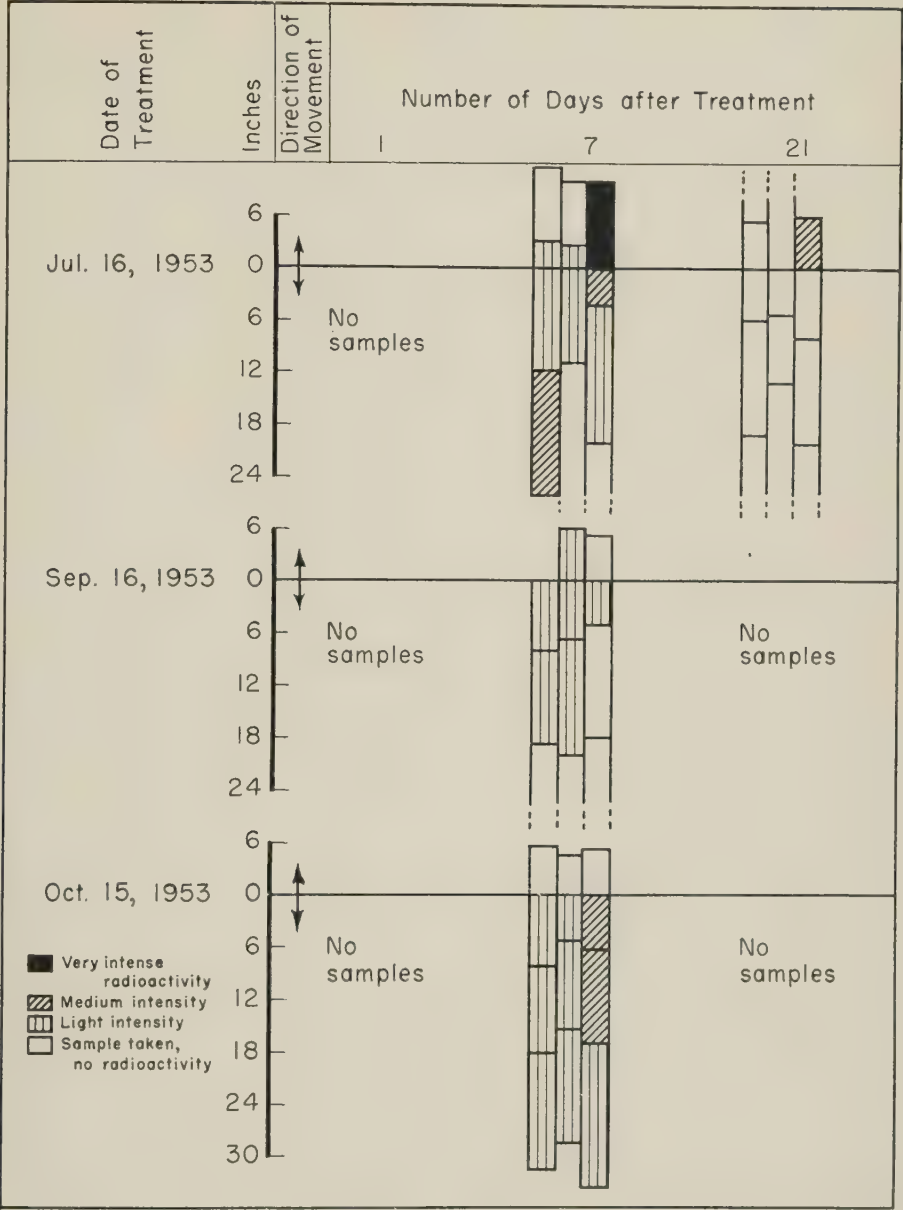


Fig. 16C. Willow: same as 16A except that treatments were later.

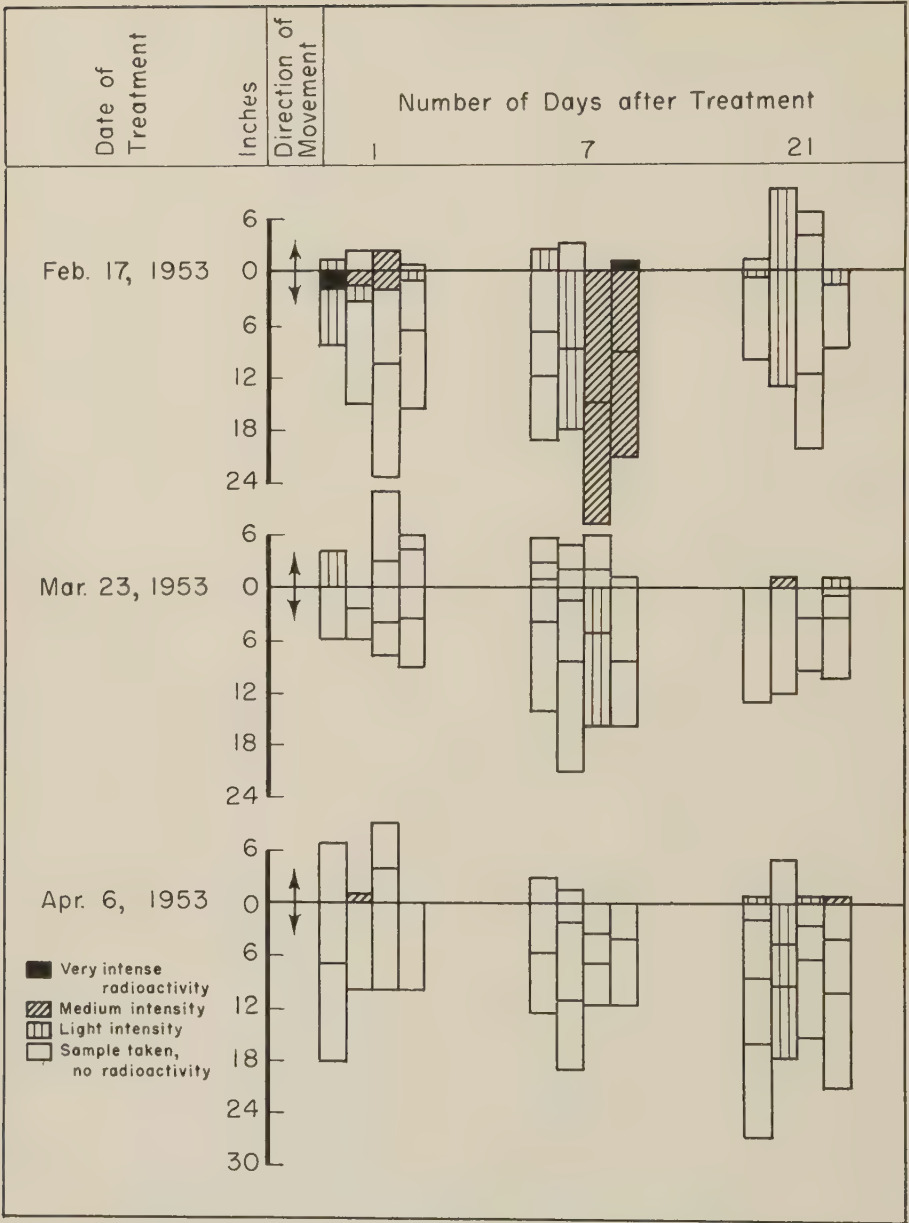


Fig. 17A. Charts showing distribution of radioactivity in shoots of *Ceanothus cuneatus* after leaves were treated with 2,4-D*. Bars above zero lines represent upward movement of the tracer; below zero, downward movement. All treated leaves were mature.

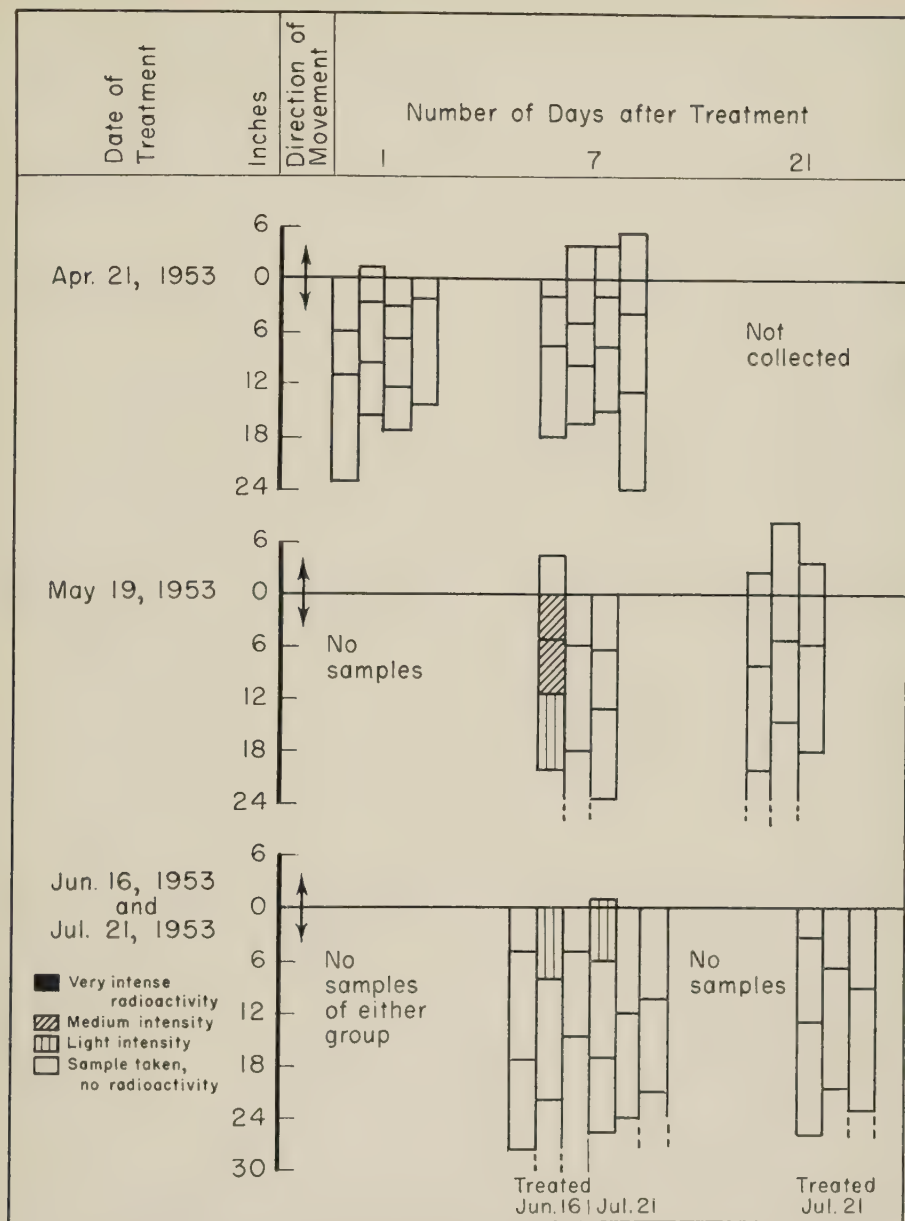


Fig. 17B. *C. cuneatus*: same as 17A except that treatments were later. All treated leaves were mature.

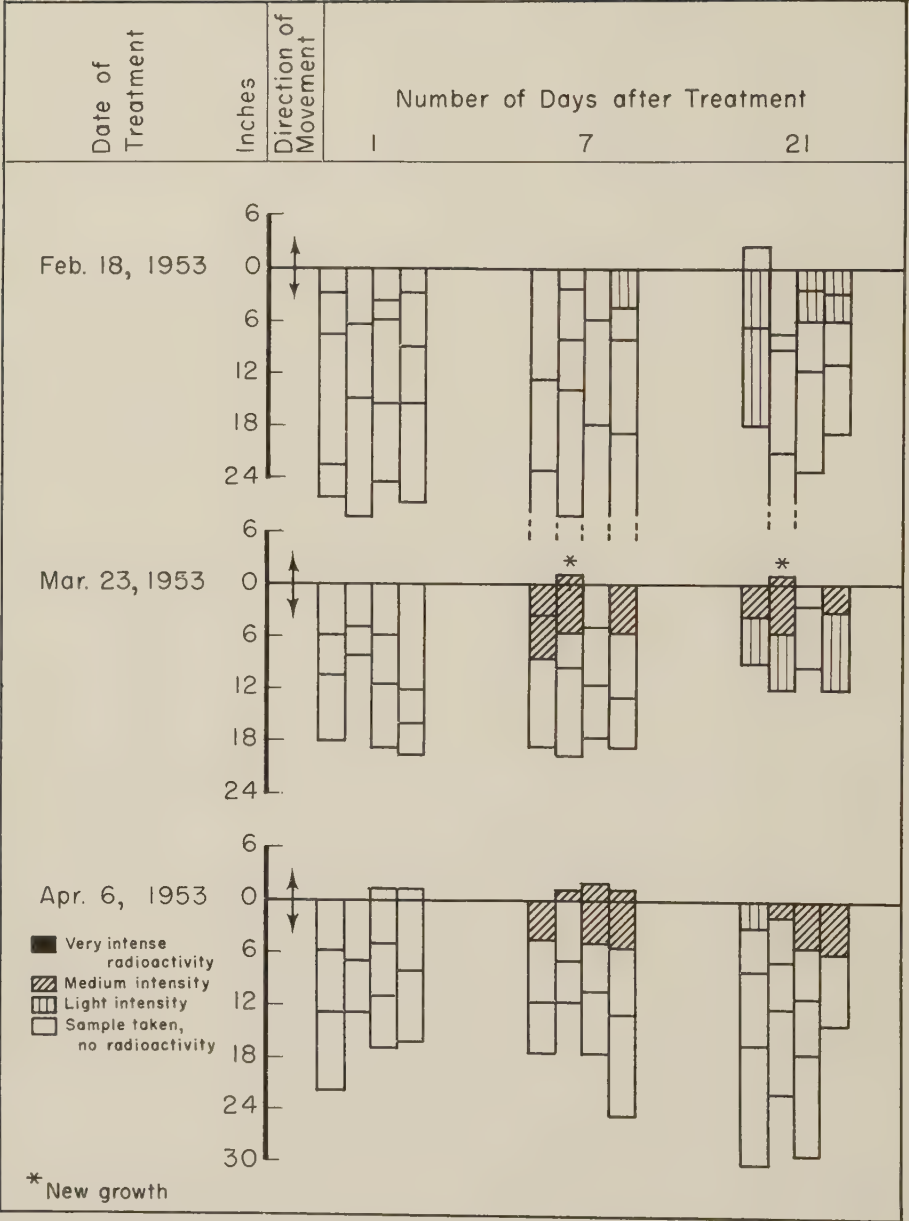


Fig. 18A. Charts showing distribution of radioactivity in shoots of *Arctostaphylos manzanita*. All treated leaves were mature.

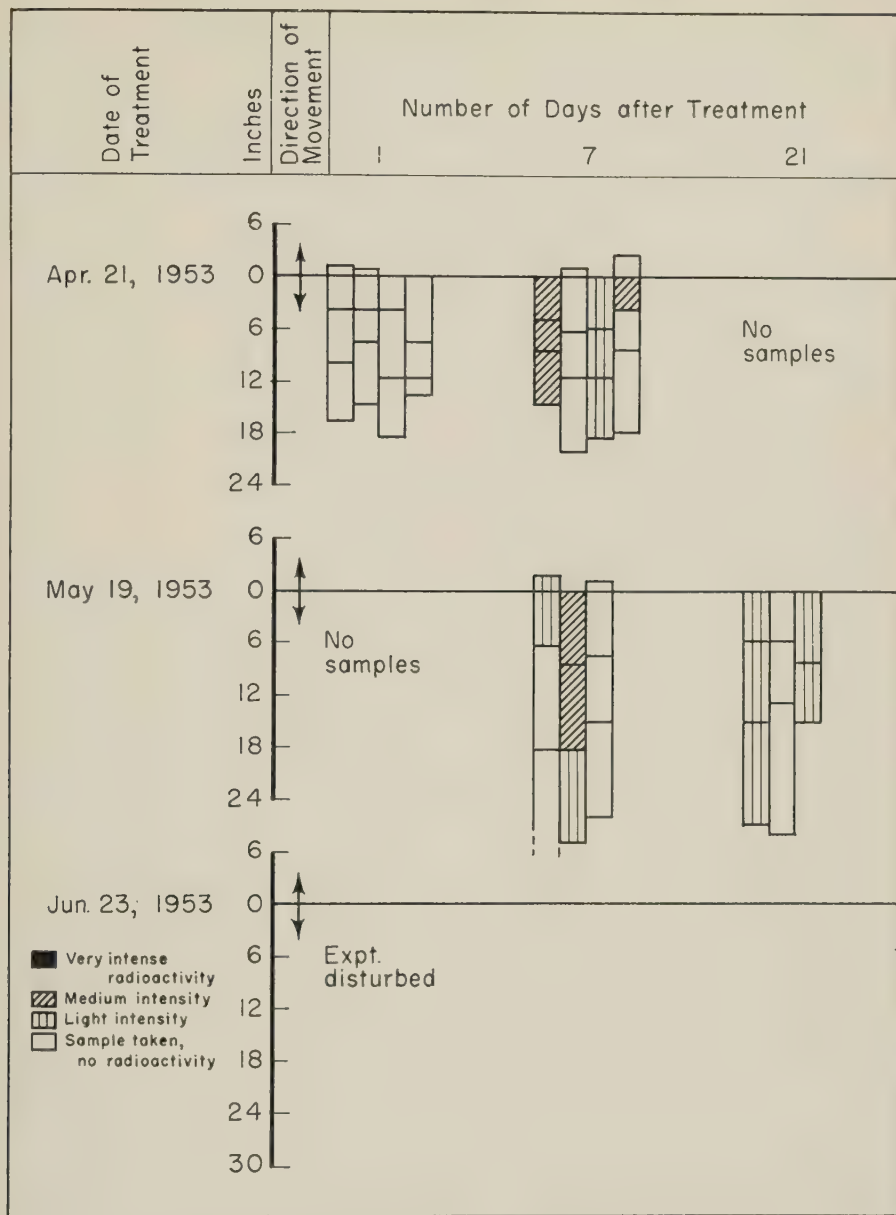


Fig. 18B. *A. manzanita*: same as 18A except that treatments were later. All treated leaves were mature.

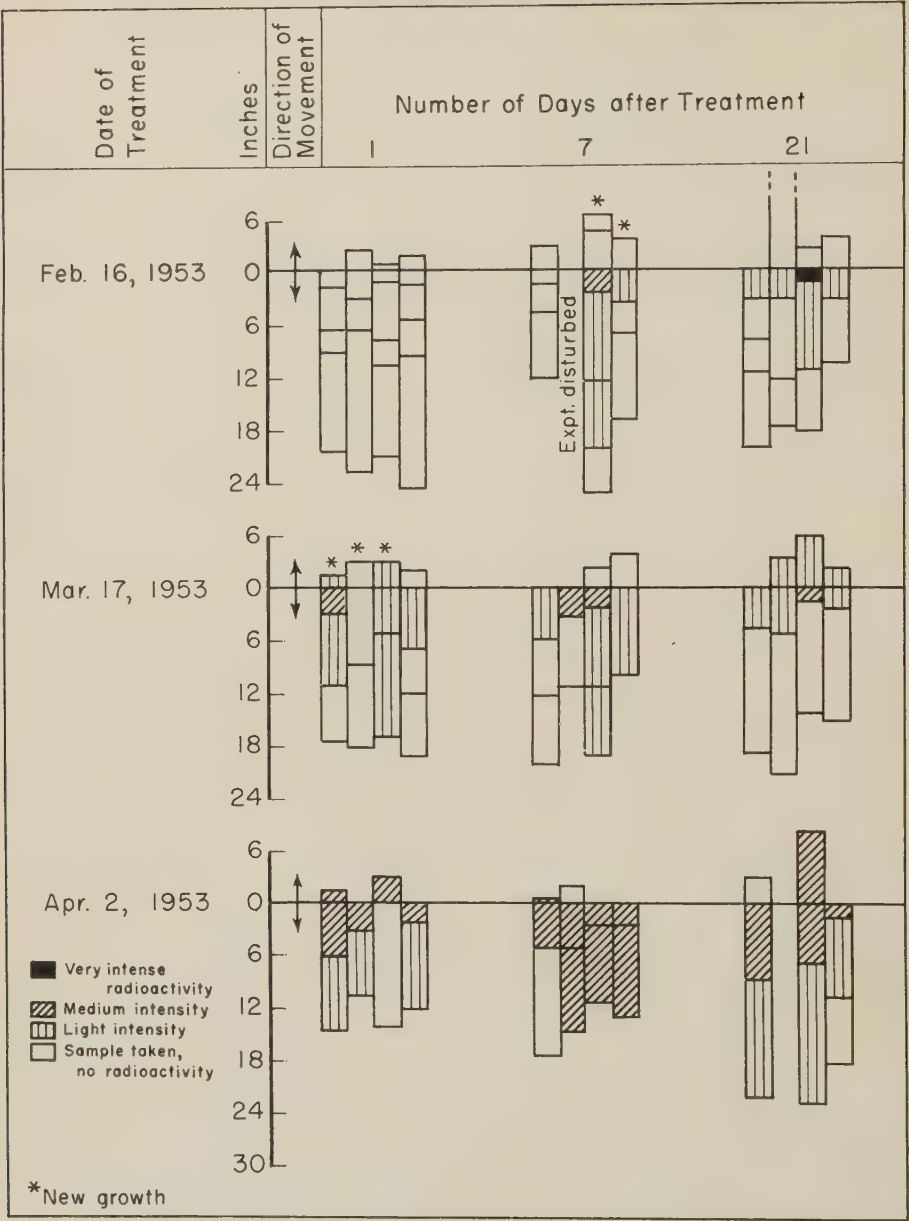


Fig. 19A. Charts showing distribution of radioactivity in shoots of toyon after leaves were treated with 2,4-D*. Bars above zero lines represent upward movement of the tracer; below zero, downward movement. All treated leaves were mature.

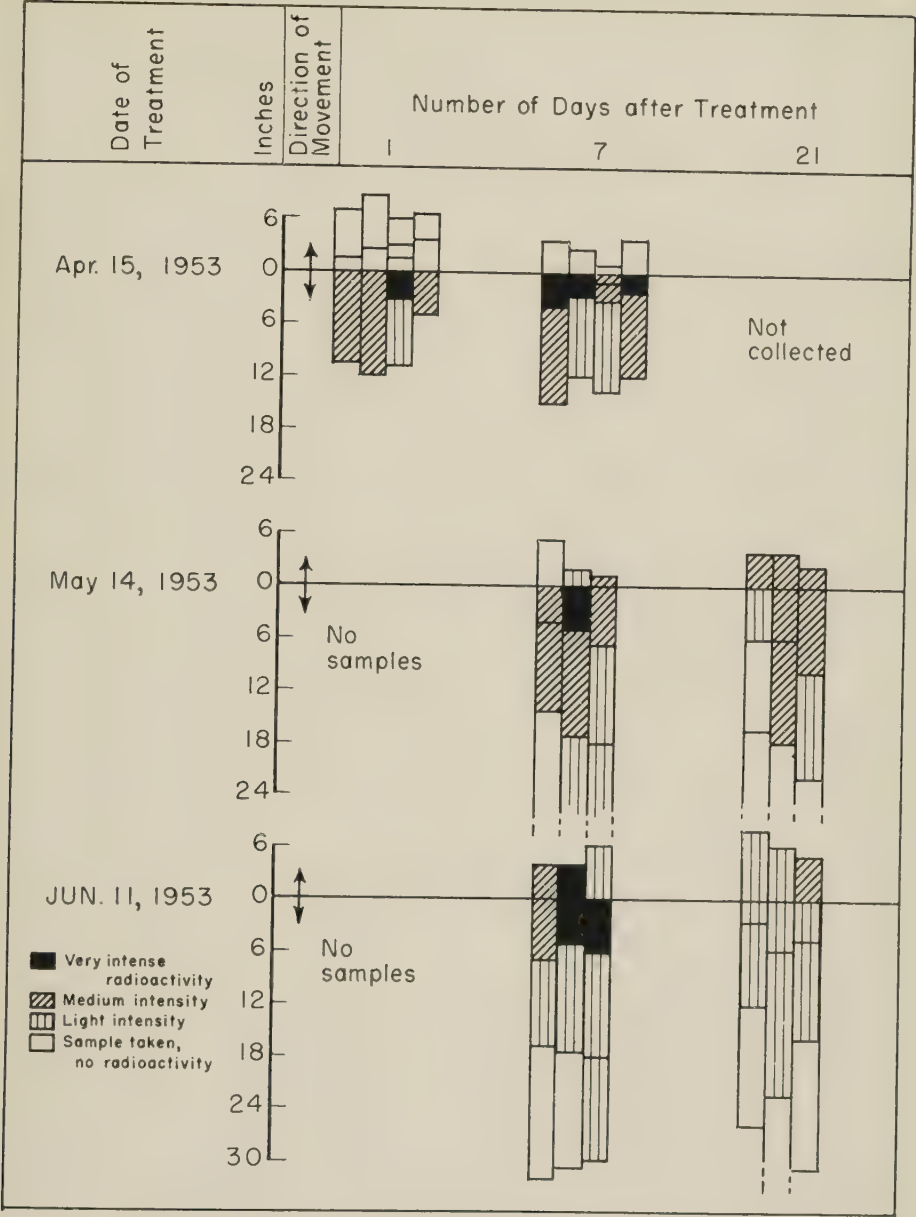


Fig. 19B. Toyon: same as 19A except that treatments were later. All treated leaves were mature.

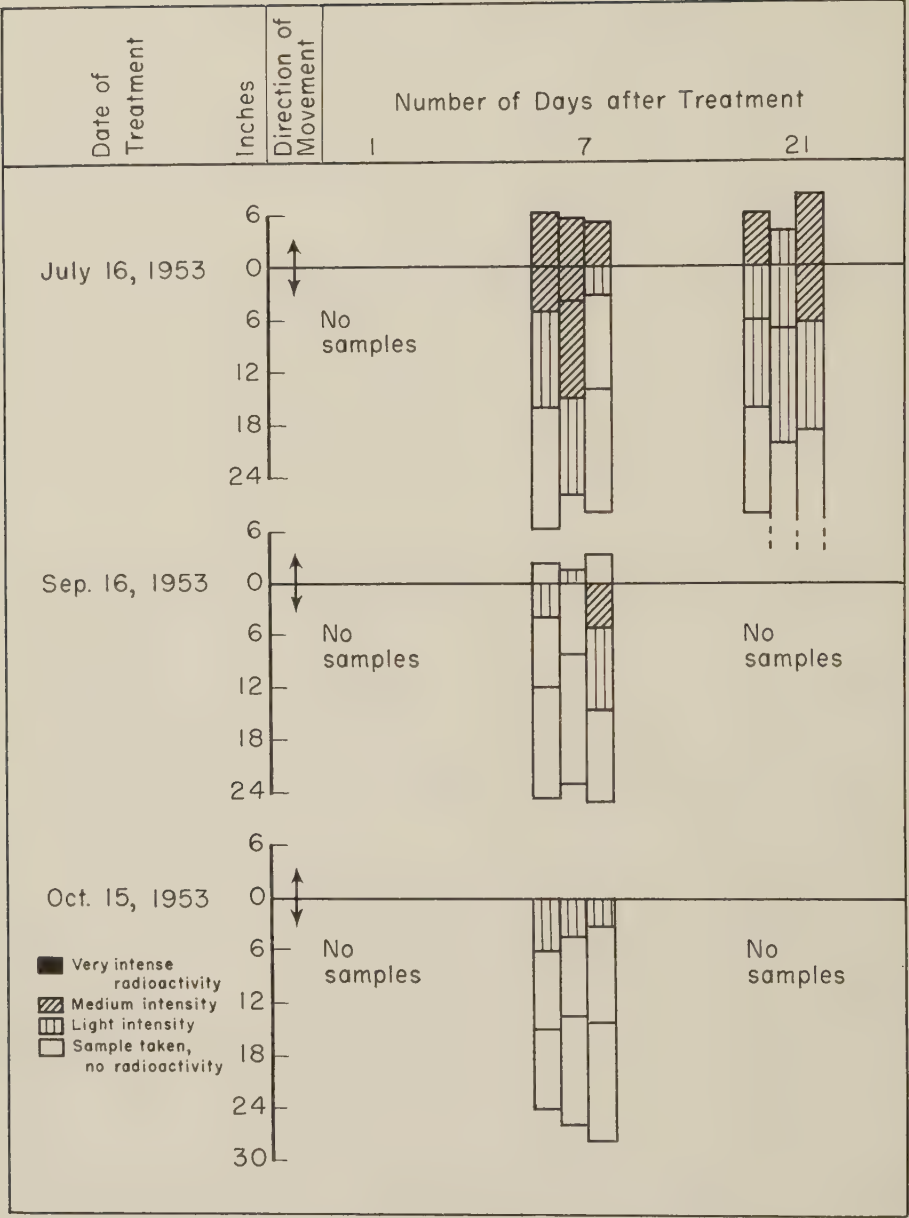


Fig. 19C. Toyon: same as 19A except that treatments were later.
All treated leaves were mature.

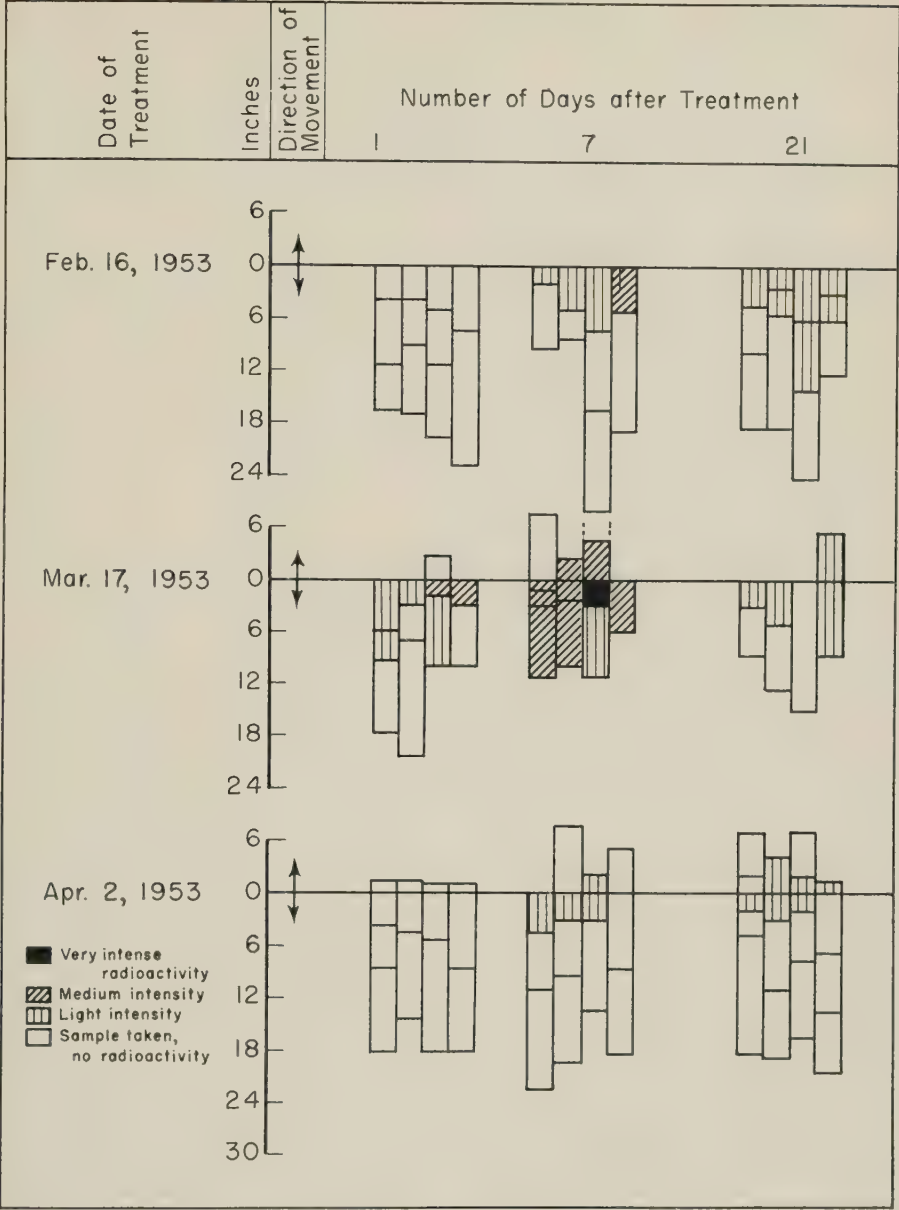


Fig. 20A. Charts showing distribution of radioactivity in shoots of live oak after leaves were treated with 2,4-D*. Bars above zero lines represent upward movement of the tracer; below zero, downward movement. All treated leaves were mature.

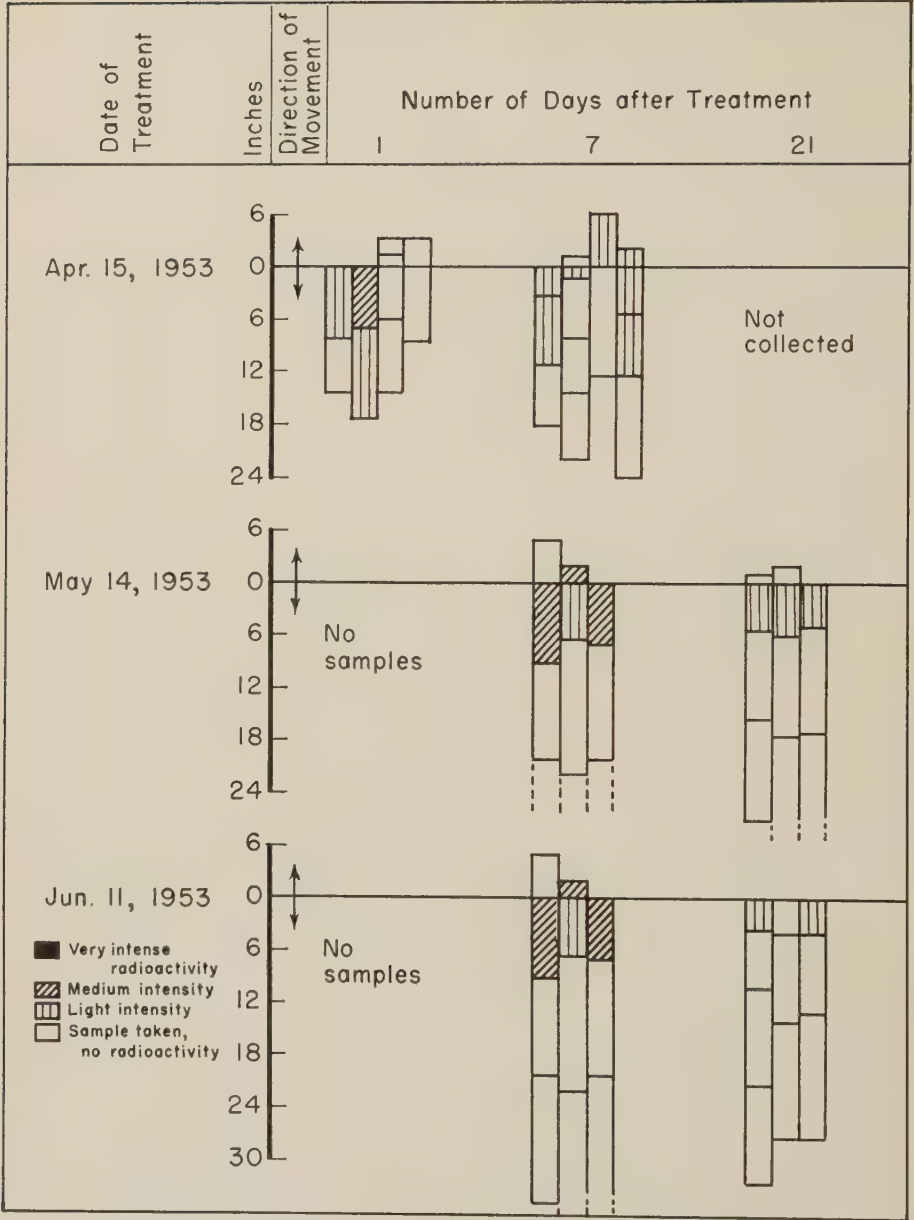


Fig. 20B. Live oak: later treatments. All treated leaves were mature.

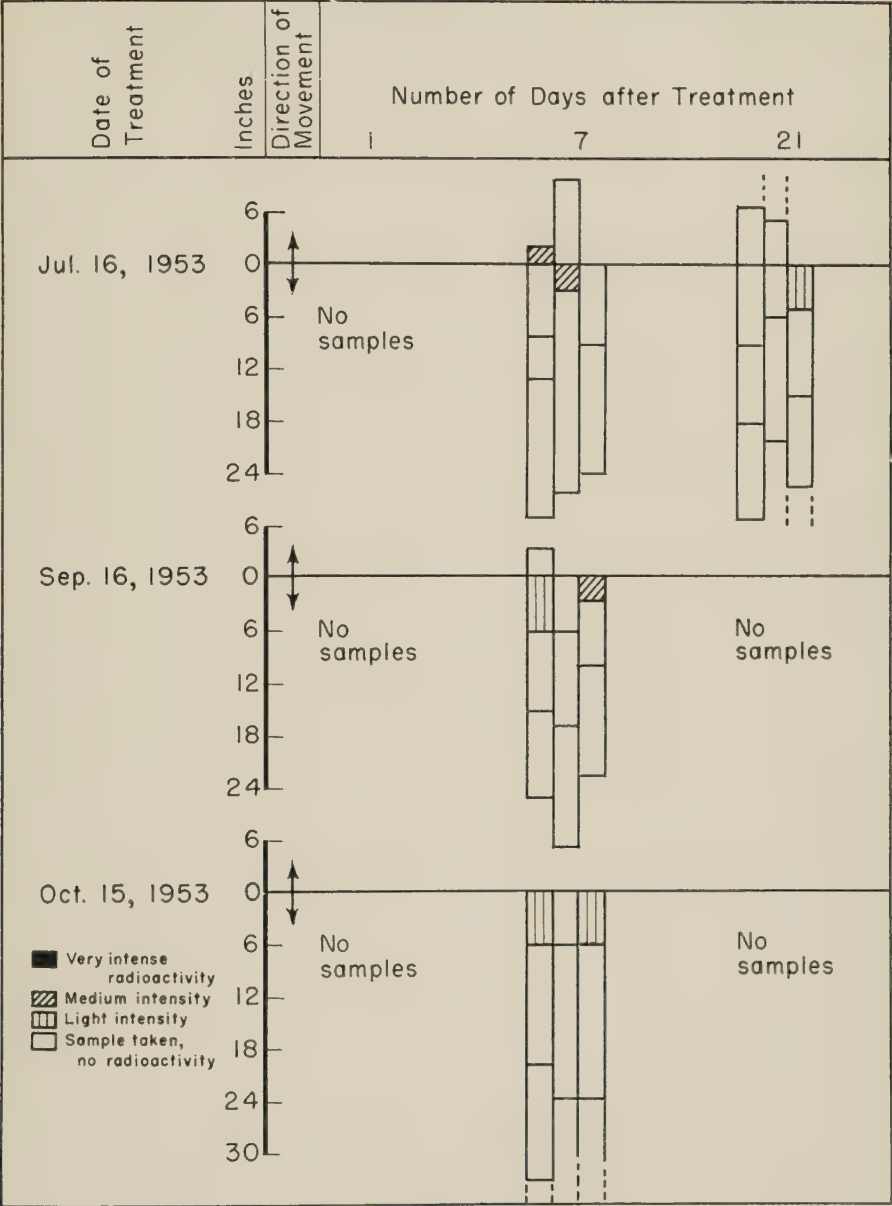


Fig. 20C. Live oak: later treatments. All treated leaves were mature.

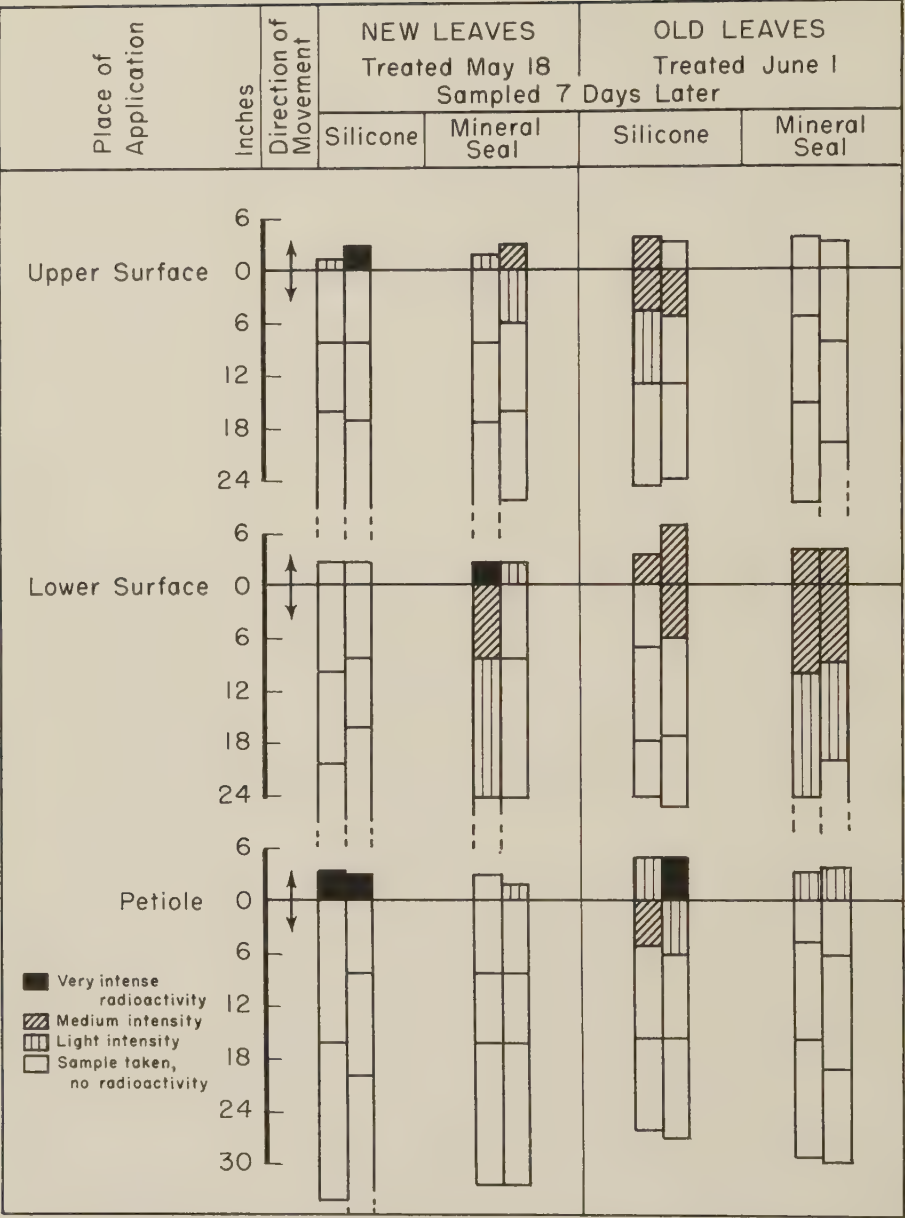


Fig. 21A. Charts showing distribution of radioactivity in shoots of toyon treated with two formulations designed to show differences in penetration. Bars above zero lines represent upward movement of the tracer; below zero, downward movement.

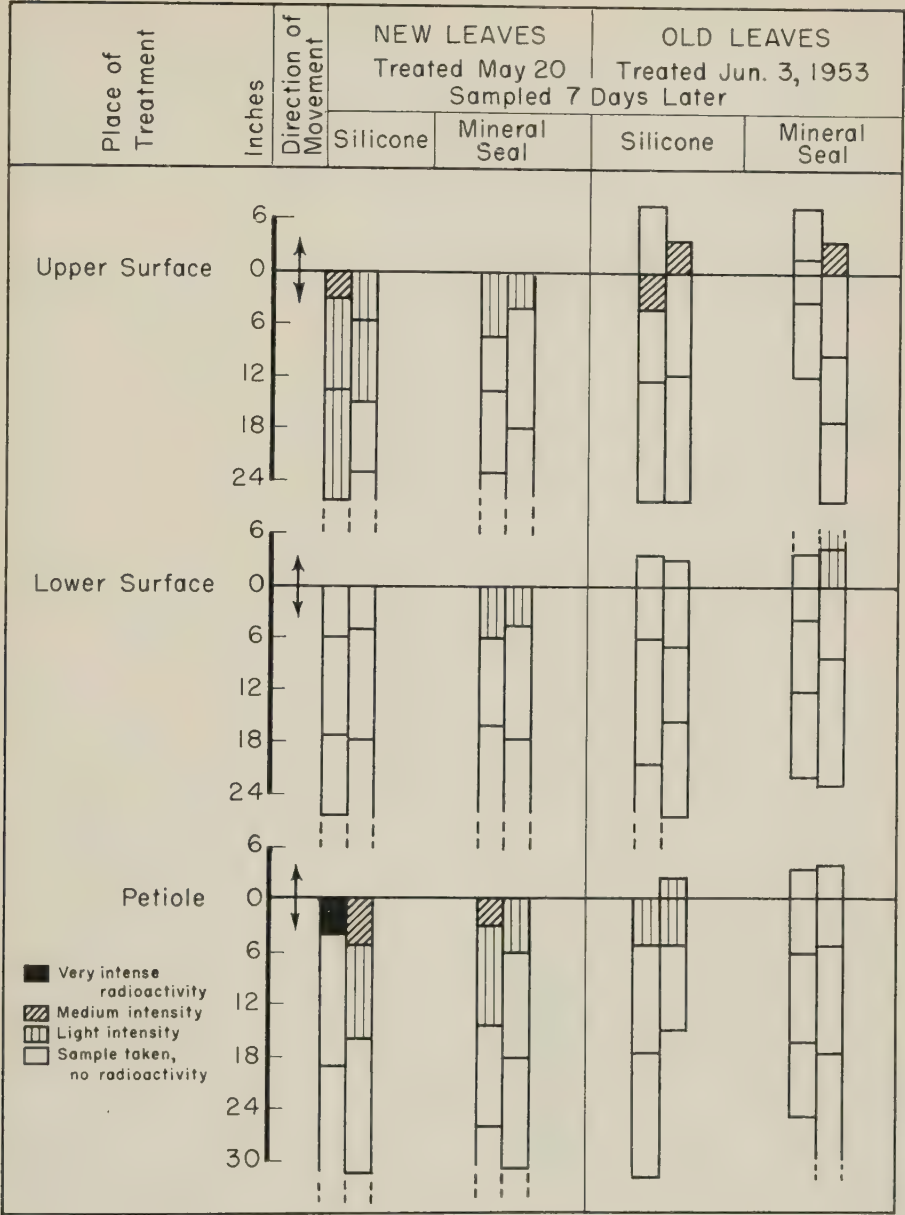


Fig. 21B. Same as 21A, but on shoots of live oak.

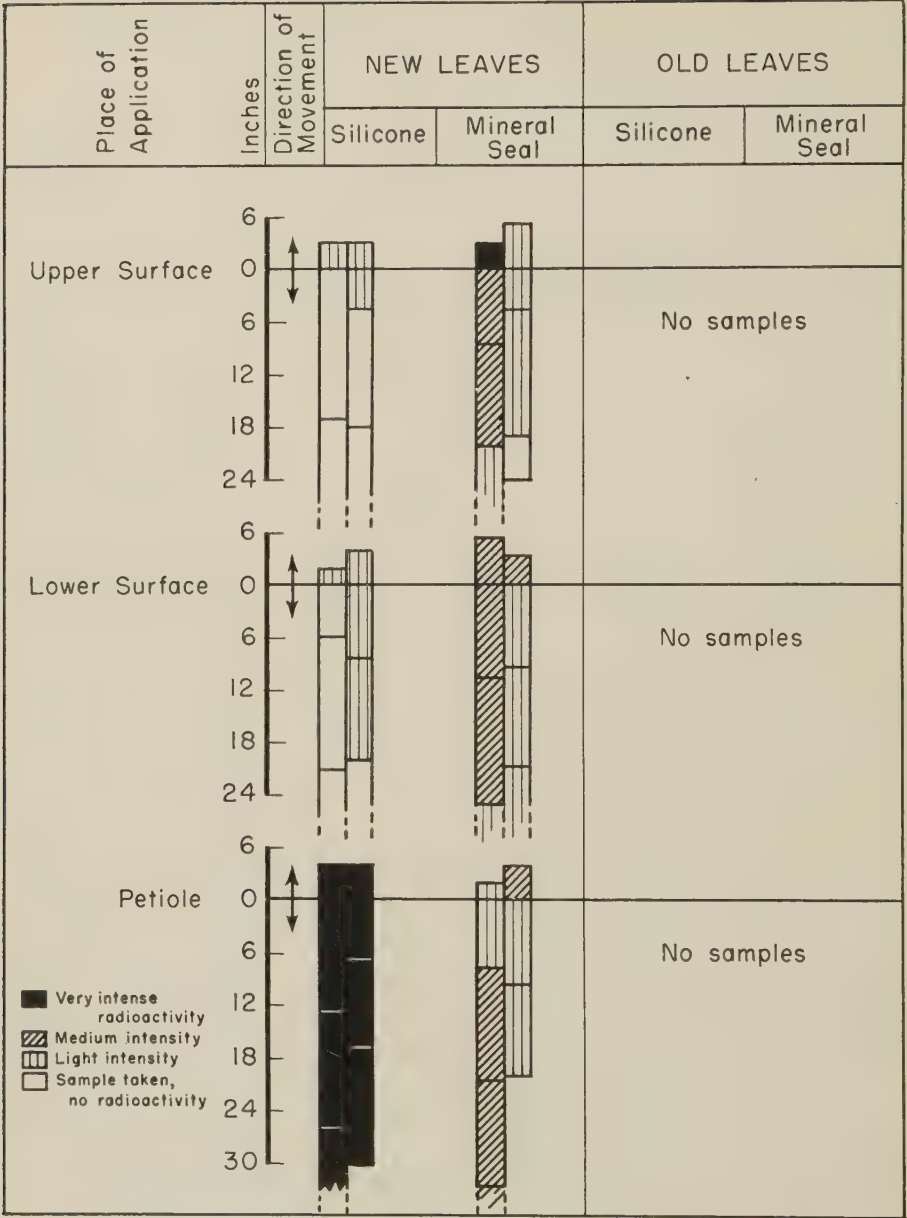


Fig. 21C. Same as 21A, but on shoots of coyote brush.

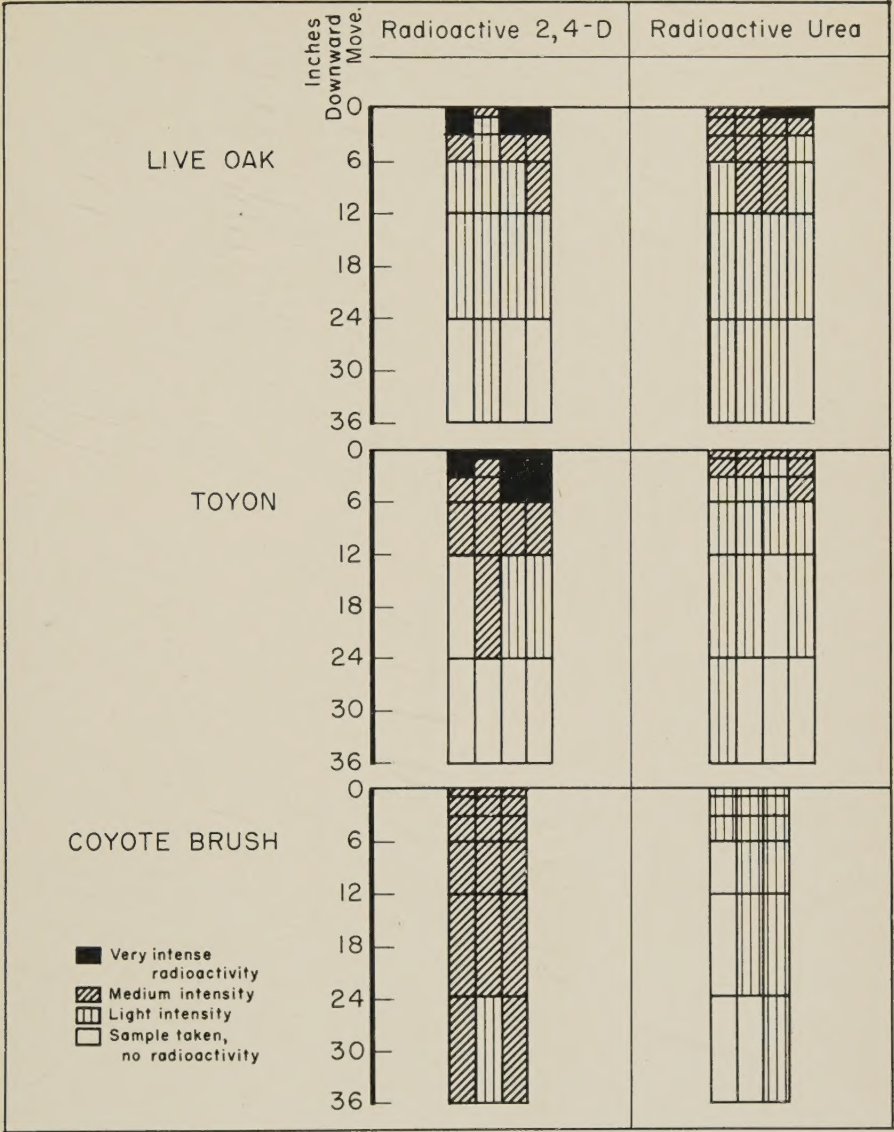


Fig. 22. Movement of radioactive 2,4-D and radioactive urea in live oak, toyon, and coyote brush. Applications were made on April 5, 1954, and samples were collected eight days later. Ten leaves were treated per branch.

